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FILE 'USPAT' ENTERED AT 12:22:19 ON 17 MAY 1999

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 *    U.S. PATENT TEXT FILE     *
 *                              *
 * THE WEEKLY PATENT TEXT AND IMAGE DATA IS CURRENT
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 * THROUGH May 11, 1999.        *
 *                              *
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=> s dhfr

L1      2071 DHFR

=> s recombinase??

'?' TRUNCATION SYMBOL NOT VALID WITHIN 'RECOMBINASE??'

=> s recombinase##

L2       203 RECOMBINASE##

=> s l1 and l2

L3        29 L1 AND L2

=> s homologous(w)recombination

      14557 HOMOLOGOUS
      14592 RECOMBINATION
L4     2074 HOMOLOGOUS(W)RECOMBINATION

=> s l1 and l4

L5       355 L1 AND L4

=> s selection(w)marker

     269541 SELECTION
      35198 MARKER
L6     1111 SELECTION(W)MARKER

=> s l5 and l6

L7       135 L5 AND L6

=> s transcription(w)factor##

      13201 TRANSCRIPTION
     444928 FACTOR##
L8     1541 TRANSCRIPTION(W)FACTOR##

=> s zinc(w)finger##

     137325 ZINC
     159155 FINGER##
L9       345 ZINC(W)FINGER##

=> s l8 or l9

L10      1696 L8 OR L9

=> s l5 and l10

L11        86 L5 AND L10

=> s enhancer## or promoter##

      13337 ENHANCER##
      35072 PROMOTER##
L12    43136 ENHANCER## OR PROMOTER##

=> d his

   (FILE 'USPAT' ENTERED AT 12:22:19 ON 17 MAY 1999)

L1      2071 S DHFR
L2       203 S RECOMBINASE##
L3        29 S L1 AND L2
L4      2074 S HOMOLOGOUS(W)RECOMBINATION
L5       355 S L1 AND L4
L6      1111 S SELECTION(W)MARKER
L7       135 S L5 AND L6
L8      1541 S TRANSCRIPTION(W)FACTOR##
L9       345 S ZINC(W)FINGER##
L10     1696 S L8 OR L9
L11       86 S L5 AND L10
L12    43136 S ENHANCER## OR PROMOTER##

=> s l2 and l12

L13      154 L2 AND L12

=> s l2(p)l12

L14       62 L2(P)L12

=> s l1(p)l4

L15       25 L1(P)L4

=> s l3 or l14 or l15

L16      100 L3 OR L14 OR L15

=> d l16,cit,rel,ab,1-100


1. 5,895,753, Apr. 20, 1999, Method for in vitro protein synthesis;
Robert Mierendorf, et al., 435/68.1, 69.1, 70.1 [IMAGE AVAILABLE]

US PAT NO:    5,895,753 [IMAGE AVAILABLE]          L16: 1 of 100
REL-US-DATA:  Continuation of Ser. No. 336,039, Nov. 8, 1994, abandoned.

ABSTRACT:
A method for performing coupled in vitro transcription and translation
reactions is disclosed In the transcription reaction, the quantity of
mRNA produced is limited to a level of less than about 2.5 micrograms in
a 10 microliter volume prior to the translation elements being added.
Limiting the level of mRNA produced prevents saturation of the
translational processes and thus aids in the efficiency and fidelity of
the translation process.

2. 5,891,668, Apr. 6, 1999, Mammalian tumor susceptibility genes and
their uses; Limin Li, et al., 435/69.1, 320.1, 357; 536/23.5 [IMAGE
AVAILABLE]

US PAT NO:    5,891,668 [IMAGE AVAILABLE]          L16: 2 of 100
REL-US-DATA:  Continuation-in-part of Ser. No. 585,758, Jan. 16, 1996.

ABSTRACT:
TSG101 is a tumor susceptibility gene whose homozygous functional knock
out in fibroblasts leads to transformation and the ability of these cells
to form metastatic tumors in nude mice. The cellular transformation that
results from inactivation of TSG101 is reversible by restoration of
TSG101 function. Decreased expression of TSG101 is associated with the
occurrence of certain human cancers, including breast carcinomas. The
TSG101 nucleic acid compositions find use in identifying homologous or
related proteins and the DNA sequences encoding such proteins; in
producing compositions that modulate the expression or function of the
protein; and in studying associated physiological pathways. In addition,
modulation of the gene activity in vivo is used for prophylactic and
therapeutic purposes, such as treatment of cancer, identification of cell
type based on expression, and the like. The DNA is further used as a
diagnostic for a genetic predisposition to cancer, and to identify
specific cancers having mutations in this gene.

3. 5,888,981, Mar. 30, 1999, Methods for regulating gene expression;
Hermann Bujard, et al., 514/44; 424/93.21 [IMAGE AVAILABLE]

US PAT NO:    5,888,981 [IMAGE AVAILABLE]          L16: 3 of 100
REL-US-DATA:  Continuation-in-part of Ser. No. 260,452, Jun. 14, 1994,
              Pat. No. 5,650,298, which is a continuation-in-part of
              Ser. No. 76,327, Jun. 14, 1993, abandoned.
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ABSTRACT:
A method for regulating expression of a tet operator-linked gene in a cell of a subject is disclosed. In one embodiment, the method involves introducing into the cell a nucleic acid molecule encoding a tetracycline-controllable transactivator (tTA), the tTA comprising a Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells; and modulating the concentration of a tetracycline, or analogue thereof, in the subject. Alternatively, in another embodiment, the method involves obtaining the cell from the subject, introducing into the cell a first nucleic acid molecule which operatively links a gene to at least one tet operator sequence, introducing into the cell a second nucleic acid molecule encoding a tTA, to form a modified cell, administering the modified cell to the is subject, and modulating the concentration of a tetracycline, or analogue thereof, in the subject. The first and second nucleic acid molecule can be within a single molecule (e.g., in the same vector) or on separate molecules.

4. 5,888,732, Mar. 30, 1999, Recombinational cloning using engineered recombination sites; James L. Hartley, et al., 435/6, 91.42, 320.1; 536/23.1, 24.2 [IMAGE AVAILABLE]

US PAT NO: 5,888,732 [IMAGE AVAILABLE] L16: 4 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 486,139, Jun. 7, 1995, abandoned.

ABSTRACT:
Recombinational cloning is provided by the use of nucleic acids, vectors and methods, in vitro and in vivo, for moving or exchanging segments of DNA molecules using engineered recombination sites and recombination proteins to provide chimeric DNA molecules that have the desired characteristic(s) and/or DNA segment(s).

5. 5,885,776, Mar. 23, 1999, Glaucoma compositions and therapeutic and diagnositic uses therefor; Edwin M. Stone, et al., 435/6 [IMAGE AVAILABLE]

US PAT NO: 5,885,776 [IMAGE AVAILABLE] L16: 5 of 100

ABSTRACT:
Methods and compositions for treating glaucoma; and glaucoma diagnostics are disclosed.

6. 5,882,888, Mar. 16, 1999, DNA integration by transposition; Steen Troels J.o slashed.rgensen, 435/69.1, 91.4, 243, 252.31, 320.1; 536/23.1, 24.2 [IMAGE AVAILABLE]

US PAT NO: 5,882,888 [IMAGE AVAILABLE] L16: 6 of 100

ABSTRACT:

Multicopy strains of gram-positive bacteria carrying multiple copies of a DNA sequence of interest may be constructed by use of a method involving introduction of a DNA construct comprising the DNA sequence of interest into the genome of the recipient cell by transposition and subsequent deletion of a marker gene used for selection of the cells having received the DNA construct by a resolution system. The multicopy strains are preferably free from a gene encoding an undesirable marker such as an antibiotic resistance marker.

7. 5,880,333, Mar. 9, 1999, Control of gene expression in plants by receptor mediated transactivation in the presence of a chemical ligand; Stephen A. Goff, et al., 800/288; 435/69.1, 69.7, 468; 514/2, 8, 21; 536/23.4, 23.5, 24.1; 800/278, 298, 320.1, 320.3 [IMAGE AVAILABLE]

US PAT NO: 5,880,333 [IMAGE AVAILABLE] L16: 7 of 100
REL-US-DATA: Continuation of Ser. No. 398,037, Mar. 3, 1995, abandoned.

ABSTRACT:
The present invention is drawn to a method of controlling gene expression in plants. Specifically, the method comprises obtaining a transgenic plant comprising at least two receptor expression cassettes and at least one target expression cassette. The first receptor expression cassette comprises a nucleotide sequence for a 5' regulatory region operably linked to a nucleotide sequence which encodes a first receptor polypeptide, and a 3' termination region. The second receptor expression cassette comprises a nucleotide sequence for a 5' regulatory region operably linked to a nucleotide sequence which encodes a second receptor polypeptide, and a 3' termination region. The target expression cassette comprises a nucleotide sequence for a 5' regulatory region operably linked to a nucleotide sequence which encodes a target polypeptide, and a 3' termination region, wherein the 5' regulatory region of said target expression cassette is activated by said first and second receptor polypeptides in the presence of one or more chemical ligands which are complementary to the ligand binding domain of said receptor polypeptides, whereby expression of said target polypeptide is accomplished. The method is useful for controlling various traits of agronomic importance, such as plant fertility.

8. 5,877,400, Mar. 2, 1999, Transgenic methods and compositions for producing parthenocarpic fruits and vegetables; Dwight T. Tomes, et al., 435/69.1; 536/23.6, 23.7, 24.1 [IMAGE AVAILABLE]

US PAT NO: 5,877,400 [IMAGE AVAILABLE] L16: 8 of 100

ABSTRACT:
The invention discloses a transgenic method for producing parthenocarpic fruits or fruits with reduced seed number. It involves the temporal expression of a plant hormone or precursor or other such gene so that gibberellin or other similar hormonal activity involved in initiating fruit set activity is potentiated. The gene is operably linked to a regulatory promoter so that expression is timed prior to pollen development or fertilization. Expression of the hormone causes fruit development in the absence of fertilization. The method also results in a fruit that has diminished or very little seed. The invention also includes transgenic constructs, vectors, and methods for production of the parthenocarpic plants.

9. 5,871,907, Feb. 16, 1999, Methods for producing members of specific binding pairs; Gregory Paul Winter, et al., 435/6, 69.1, 91.1, 235.1, 320.1 [IMAGE AVAILABLE]

US PAT NO: 5,871,907 [IMAGE AVAILABLE] L16: 9 of 100

ABSTRACT:

The invention provides methods and kits for producing specific binding pairs (sbp) members. Populations of polypeptide chain components of sbp members are combined to form libraries of sbps displayed by secreted replicable genetic display packages (rgdp). At least one of the polypeptide chains is expressed as a fusion with a component of an rgdp which thereby displays that polypeptide chain at the surface of rgdp. At least one population of polypeptide chains is expressed from nucleic acid which is capable of being packaged using a component of an rgdp, whereby the genetic material of rgdps produced encodes a polypeptide chain. The methods enable production of libraries of multimeric sbp members from a very large number of possible combinations. In one embodiment of the invention a method employs "chain shuffling" in the production of sbp members of desired specificity for a counterpart sbp member. Selection procedures are also described.

10. 5,869,046, Feb. 9, 1999, Altered polypeptides with increased half-life; Leonard G. Presta, et al., 424/133.1, 153.1; 530/387.3, 388.15, 388.7, 391.1 [IMAGE AVAILABLE]

US PAT NO: 5,869,046 [IMAGE AVAILABLE] L16: 10 of 100

ABSTRACT:
Polypeptides that are cleared from the kidney and do not contain in their original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding epitope of an Fc region of an IgG and thereby have increased circulatory half-life.

11. 5,866,755, Feb. 2, 1999, Animals transgenic for a tetracycline-regulated transcriptional inhibitor; Hermann Bujard, et al., 800/9, 18 [IMAGE AVAILABLE]

US PAT NO: 5,866,755 [IMAGE AVAILABLE] L16: 11 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 383,754, Feb. 3, 1995, Pat. No. 5,789,156, Ser. No. 275,876, Jul. 15, 1994, Pat. No. 5,654,168, Ser. No. 260,452, Jun. 14, 1994, Pat. No. 5,650,298, and Ser. No. 76,726, Jun. 14, 1993, Pat. No. 5,464,758, said Ser. No. 275,876 is a continuation-in-part of Ser. No. 270,637, Jul. 1, 1994, abandoned, said Ser. No. 260,452 is a continuation-in-part of Ser. No. 76,327, Jun. 14, 1993, abandoned.

ABSTRACT:
Transgenic animals carrying a transgene comprising a nucleic acid molecule encoding protein useful for regulating the expression of genes in eukaryotic cells and organisms in a highly controlled manner are disclosed. In the regulatory system of the invention, transcription of a tet operator-linked nucleotide sequence is inhibited by a transcriptional inhibitor fusion protein composed of two polypeptides, a first polypeptide which binds to tet operator sequences and a second polypeptide which directly or indirectly inhibits transcription in eukaryotic cells. In various embodiment, the first polypeptide binds to tet operator sequences either: (i) in the absence but not the presence of tetracycline (or an analogue thereof) or (ii) in the presence but not the absence of tetracycline (or an analogue thereof). In a preferred embodiment, the transgene encoding the transcriptional inhibitor fusion protein is integrated at a predetermined location within the chromosome of the transgenic animal.

12. 5,864,020, Jan. 26, 1999, HTK ligand; Brian D. Bennett, et al., 530/388.24; 435/188; 530/387.1, 391.1, 391.3 [IMAGE AVAILABLE]

US PAT NO: 5,864,020 [IMAGE AVAILABLE] L16: 12 of 100
REL-US-DATA: Division of Ser. No. 277,722, Jul. 20, 1994.

ABSTRACT:
A novel hepatoma transmembrane kinase receptor ligand (Htk ligand) which binds to, and activates, the Htk receptor is disclosed. As examples, mouse and human Htk ligands have been identified in a variety of tissues using a soluble Htk-Fc fusion protein. The ligands have been cloned and sequenced. The invention also relates to nucleic acids encoding the ligand, methods for production and use of the ligand, and antibodies directed thereto.

13. 5,859,312, Jan. 12, 1999, Transgenic non-human animals having targeting endogenous lymphocyte transduction genes and cognate human transgenes; Daniel Littman, et al., 800/9; 435/7.1; 536/23.1; 800/18 [IMAGE AVAILABLE]

US PAT NO: 5,859,312 [IMAGE AVAILABLE] L16: 13 of 100
REL-US-DATA: Continuation of Ser. No. 590,051, Jan. 3, 1996, abandoned, which is a continuation of Ser. No. 943,818, Sep. 11, 1992, abandoned.

ABSTRACT:
The invention provides transgenic non-human animals and transgenic non-human mammalian cells having at least one functionally disrupted lymphocyte transduction locus, particularly a CD4 locus, targeting constructs used to produce such transgenic stem cells and animals, methods and targeting constructs for inactivating or suppressing expression of endogenous lymphocyte transduction gene loci, transgenes encoding heterologous lymphocyte transduction proteins, and nonhuman animals that express a human lymphocyte transduction protein and lack expression of a cognate murine lymphocyte transduction protein.

14. 5,859,310, Jan. 12, 1999, Mice transgenic for a tetracycline-controlled transcriptional activator; Hermann Bujard, et al., 800/9; 435/69.1, 70.1, 320.1, 325; 514/152; 536/23.4, 24.1; 800/4, 18, 22, 25 [IMAGE AVAILABLE]

US PAT NO: 5,859,310 [IMAGE AVAILABLE] L16: 14 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 260,452, Jun. 14, 1994, Pat. No. 5,650,298, which is a continuation-in-part of Ser. No. 76,327, Jun. 14, 1993, abandoned.

ABSTRACT:
Transgenic mice carrying two transgenes, the first coding for a transactivator fusion protein comprising a tet repressor and a polypeptide which directly or indirectly activates transcription of a tet operator-linked gene in eucaryotic cells, and the second comprising a gene operably linked to a minimal promotor operably linked to at least one tet operator sequence, are disclosed. Isolated DNA molecules (e.g., targeting vectors) for integrating a polynucleotide sequence encoding a transactivator of the invention at a predetermined location within a second target DNA molecule by homologous recombination are also disclosed. Transgenic mice having the DNA molecules of the invention integrated at a predetermined location in a chromosome by homologous recombination are also encompassed by the invention. Methods to regulate the expression of a tet operator linked-gene of interest by administering tetracycline or a tetracycline analogue to a mouse of the invention are also disclosed. The regulatory system of the invention allows for conditional inactivation or modulation of expression of a gene of interest in a host cell or mouse.

15. 5,859,307, Jan. 12, 1999, Mutant RAG-1 deficient animals having no mature B and T lymphocytes; Peter Mombaerts, et al., 800/9; 424/9.1, 9.2; 800/3, 10, 11, 18, 21, 22, 25 [IMAGE AVAILABLE]

US PAT NO: 5,859,307 [IMAGE AVAILABLE] L16: 15 of 100
REL-US-DATA: Continuation of Ser. No. 830,831, Feb. 4, 1992, abandoned.

ABSTRACT:
Immunodeficient animals are generated by introducing a mutation in RAG-1 into the germline of the animals via gene targeting in embryonic stem cells. The production of mutant RAG-1 deficient mice is detailed. RAG-1 deficient mice have no mature B and T lymphocytes. The arrest of B and T cell differentiation occurs at an early stage and correlates with the inability to perform V(D)J recombination. To date, these mice do not have mature B and T lymphocytes, nor do they express immunoglobulin or T cell receptors. The same strategy can be applied to the generation of other RAG-1 deficient animals, such as rabbits, rats, and pigs, using known techniques. These animals are all useful for the same general purposes as the scid mice, for example, cultivation of human lymphocytes for expression of human immunoglobulin. Other uses include the establishment of continuous lymphoid cell lines and, by crossbreeding with other lines of animals, the establishment of animals developing tumors for use in studying tumor cell developments and treatments.

16. 5,858,657, Jan. 12, 1999, Methods for producing members of specific binding pairs; Gregory Paul Winter, et al., 435/6, 69.6, 91.1 [IMAGE AVAILABLE]

US PAT NO: 5,858,657 [IMAGE AVAILABLE] L16: 16 of 100
REL-US-DATA: Continuation of Ser. No. 150,002, Mar. 31, 1994.

ABSTRACT:
The invention provides methods and kits for producing specific binding pairs (sbp) members. Populations of polypeptide chain components of sbp members are combined to form libraries of sbps displayed by secreted replicable genetic display packages (rgdp). At least one of the polypeptide chains is expressed as a fusion with a component of an rgdp which thereby displays that polypeptide chain at the surface of rgdp. At least one population of polypeptide chains is expressed from nucleic acid which is capable of being packaged using a component of an rgdp, whereby the genetic material of rgdps produced encodes a polypeptide chain. The methods enable production of libraries of multimeric sbp members from a very large number of possible combinations. In one embodiment of the invention a method employs "chain shuffling" in the production of sbp members of desired specificity for a counterpart sbp member. Selection procedures are also described.

17. 5,851,808, Dec. 22, 1998, Rapid subcloning using site-specific recombination; Stephen J. Elledge, et al., 435/91.4, 91.41, 320.1; 536/23.1 [IMAGE AVAILABLE]

US PAT NO: 5,851,808 [IMAGE AVAILABLE] L16: 17 of 100

ABSTRACT:
The present invention provides compositions, including vectors, and methods for the rapid subcloning of nucleic acid sequences in vivo and in vitro. In particular, the invention provides vectors used to contain a gene of interest that comprise a sequence-specific **recombinase** target site. These vectors are used to rapidly transfer the gene of interest into any expression vector that contains a sequence-specific **recombinase** target site located downstream of a **promoter** element so that the gene of interest may be expressed.

18. 5,851,796, Dec. 22, 1998, Autoregulatory tetracycline-regulated system for inducible gene expression in eucaryotes; David G. Schatz, 435/69.1, 320.1, 325, 410; 536/23.4 [IMAGE AVAILABLE]

US PAT NO: 5,851,796 [IMAGE AVAILABLE] L16: 18 of 100

ABSTRACT:
A tetracycline-regulated system which provides autoregulatory, inducible gene expression in cultured cells and transgenic animals is described. In the autoregulatory plasmid pTet-tTAk, a modified tTA gene called tTAk was placed under the control of Tetp. Tetracycline prevents tTA from binding to Tetp, preventing expression of both tTA and luciferase. This negative feedback cycle ensures that little or no tTA is produced in the presence

of tetracycline, thereby reducing or eliminating possible toxic effects. When tetracycline is removed, however, this strategy predicts that tiny amounts of tTA protein (which may result from the leakiness of the minimal promoter), will bind to Tet-op and stimulate expression of the tTAk gene. A positive feedforward loop is initiated which in turn leads to higher levels of expression of tTA and thus, luciferase. Polynucleotide molecules encoding the autoregulatory system, as well as methods of enhancing or decreasing the expression of desired genes, and kits for carrying out these methods are described.

19. 5,849,991, Dec. 15, 1998, Mice homozygous for an inactivated .alpha. 1,3-galactosyl transferase gene; Anthony J. F. d'Apice, et al., 800/8; 435/320.1, 354, 463; 800/17, 18, 21, 22, 24 [IMAGE AVAILABLE]

US PAT NO: 5,849,991 [IMAGE AVAILABLE] L16: 19 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 188,607, Jan. 27, 1994, abandoned.

ABSTRACT:
Human pre-formed xenoantibodies play an important role in the hyperacute rejection response in human xenotransplantation. Disclosed are materials and methods for removing or neutralizing such antibodies. Also disclosed are materials and methods for reducing or eliminating the epitopes in the donor organs that are recognized by such antibodies. Such epitopes are formed as the result of activity by the enzyme .alpha.-1,3 galactosyltransferase. The porcine gene encoding .alpha.-1,3 galactosyltransferase is disclosed, as are materials and methods for inactivating ("knocking out") the .alpha.-1,3 galactosyltransferase gene in mammalian cells and embryos. Included are nucleic acid constructs useful for inactivating the .alpha.-1,3 galactosyltransferase gene in a target cell. Also disclosed is a novel leukemia inhibitory factor (T-LIF) that is useful for maintenance of embryonic stem cells and primordial germ cells in culture.

20. 5,849,989, Dec. 15, 1998, Insulin promoter factor, and uses related thereto; Thomas Edlund, 800/9, 18 [IMAGE AVAILABLE]

US PAT NO: 5,849,989 [IMAGE AVAILABLE] L16: 20 of 100

ABSTRACT:
The present invention relates to the discovery in eukaryotic cells, particularly mammalian cells, of novel a transcriptional regulatory factor, referred to hereinafter as "Insulin Promoter Factor 1" or "Ipf1".

21. 5,849,708, Dec. 15, 1998, Promotion of eating behavior; Eleftheria Maratos-Flier, 514/13; 530/300, 317 [IMAGE AVAILABLE]

US PAT NO: 5,849,708 [IMAGE AVAILABLE] L16: 21 of 100

ABSTRACT:
A method for promoting eating, the gain of weight or maintenance of weight in a subject. The method includes administering to the subject an effective amount of melanocyte concentrating hormone (MCH) or agonist thereof.

22. 5,849,553, Dec. 15, 1998, Mammalian multipotent neural stem cells; David J. Anderson, et al., 435/467, 69.1, 320.1, 325, 353, 368, 455, 462 [IMAGE AVAILABLE]

US PAT NO: 5,849,553 [IMAGE AVAILABLE] L16: 22 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 188,286, Jan. 28, 1994, Pat. No. 5,654,183, which is a continuation-in-part of Ser. No. 969,088, Oct. 29, 1992, abandoned, which is a continuation-in-part of Ser. No. 920,617, Jul. 27, 1992, abandoned.

ABSTRACT:
The invention includes mammalian multipotent neural stem cells and their progeny and methods for the isolation and clonal propagation of such cells. At the clonal level the stem cells are capable of self regeneration and asymmetrical division. Lineage restriction is demonstrated within developing clones which are sensitive to the local environment. The invention also includes such cells which are transfected with foreign nucleic acid, e.g., to produce an immortalized neural stem cell, and immortalized cell lines which are capable of subsequent disimmortalization. The invention further includes transplantation assays which allow for the identification of mammalian multipotent neural stem cells from various tissues and methods for transplanting mammalian neural stem cells and/or neural or glial progenitors into mammals. A novel method for detecting antibodies to neural cell surface markers is disclosed as well as a monoclonal antibody to mouse LNGFR.

23. 5,844,079, Dec. 1, 1998, Vertebrate embryonic pattern-inducing proteins, and uses related thereto; Philip W. Ingham, et al., 530/350; 435/7.1, 69.1, 252.3, 320.1; 530/300; 536/23.1, 23.5 [IMAGE AVAILABLE]

US PAT NO: 5,844,079 [IMAGE AVAILABLE] L16: 23 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 176,427, Dec. 30, 1993.

ABSTRACT:
The present invention concerns the discovery that proteins encoded by a family of vertebrate genes, termed here hedgehog-related genes, comprise morphogenic signals produced by embryonic patterning centers, and are involved in the formation of ordered spatial arrangements of differentiated tissues in vertebrates. The present invention makes available compositions and methods that can be utilized, for example to generate and/or maintain an array of different vertebrate tissue both in vitro and in vivo.

24. 5,843,744, Dec. 1, 1998, Bacillus thuringiensis Tn5401 proteins; James A. Baum, 435/183, 196 [IMAGE AVAILABLE]

US PAT NO: 5,843,744 [IMAGE AVAILABLE] L16: 24 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 89,986, Jul. 8, 1993, Pat. No. 5,441,884.

ABSTRACT:
A transposable element, or transposon, isolated from Bacillus thuringiensis (B.t.) and designated as transposon Tn5401. The invention also includes a method of using this transposon in a site-specific recombination system for construction of recombinant B.t. strains that contain insecticidal B.t. toxin protein genes and that are free of DNA not native to B.t.

25. 5,843,742, Dec. 1, 1998, Adeno-associated derived vector systems for gene delivery and integration into target cells; Georges Natsoulis, et al., 435/465, 366, 367, 369, 455 [IMAGE AVAILABLE]

US PAT NO: 5,843,742 [IMAGE AVAILABLE] L16: 25 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 357,503, Dec. 16, 1994, abandoned.

ABSTRACT:
A novel nucleotide sequence integration and targeting system is described. The system employs adeno-associated virus (AAV) derived vectors which include a selected nucleotide sequence bounded by AAV inverted terminal repeats (ITRs). An AAV rep coding region is also provided, enabling the targeted integration of the selected nucleotide sequence into the genome of a suitable target cell. The nucleotide sequence integration system of the present invention can deliver and integrate large segments of DNA into the genome of target cells. Further, the subject integration system provides the advantage of site-specific integration of the selected nucleotide sequences in a target cell genome, thereby avoiding insertional mutagenesis events experienced with prior systems.

26. 5,843,694, Dec. 1, 1998, Methods for identification of modulatory compounds for the expression of the NES1 protein; Vimla Band, 435/23, 212, 219, 226 [IMAGE AVAILABLE]

US PAT NO: 5,843,694 [IMAGE AVAILABLE] L16: 26 of 100
REL-US-DATA: Division of Ser. No. 467,155, Jun. 6, 1995.

ABSTRACT:
The expression and purification of normal epithelial specific polypeptide (NES 1) which is expressed in normal cells, but not in radiation transformed cells, are described. Both the DNA sequence encoding the NES1 and the corresponding amino acid sequence are disclosed. Also, disclosed are methods for carcinoma detection and treatment using the NES1 as well as methods of identifying compounds modulating the expression and activity of NES1.

27. 5,840,540, Nov. 24, 1998, Nucleic acids encoding presenilin II; Peter H. St. George-Hyslop, et al., 435/69.1, 252.3, 320.1, 325; 530/350; 536/23.1, 24.3 [IMAGE AVAILABLE]

US PAT NO: 5,840,540 [IMAGE AVAILABLE] L16: 27 of 100
REL-US-DATA: Division of Ser. No. 592,541, Jan. 26, 1996, which is a

continuation-in-part of Ser. No. 509,359, Jul. 31, 1995, which is a continuation-in-part of Ser. No. 496,841, Jun. 28, 1995, which is a continuation-in-part of Ser. No. 431,048, Apr. 28, 1995.

ABSTRACT:
The present invention describes the identification, isolation and cloning of two human presenilin genes, PS-1 and PS-2, mutations in which lead to Familial Alzheimer's Disease. Also identified are presenilin homologue genes in mice, C. elegans and D. melanogaster. Transcripts and products of these genes are useful in detecting and diagnosing Alzheimer's disease, developing therapeutics for treatment of Alzheimer's disease, as well as the isolation and manufacture of the protein and the constructions of transgenic animals expressing the mutant genes.

28. 5,837,844, Nov. 17, 1998, CAIP-like gene family; Yen-Ming Hsu, 536/23.5; 435/69.1, 252.3, 254.11, 320.1, 325; 530/300, 350, 351; 536/23.1 [IMAGE AVAILABLE]

US PAT NO: 5,837,844 [IMAGE AVAILABLE] L16: 28 of 100

ABSTRACT:
An isolated nucleic acid encoding a CD2 Associated Intracellular Protein (CAIP) and uses thereof.

29. 5,833,993, Nov. 10, 1998, Feline immunodeficiency virus vaccine; Richard C. Wardley, et al., 424/208.1, 199.1; 435/5, 69.7, 320.1; 530/388.35; 536/23.1 [IMAGE AVAILABLE]

US PAT NO: 5,833,993 [IMAGE AVAILABLE] L16: 29 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 236,429, Apr. 29, 1995, abandoned.

ABSTRACT:

Disclosed are vaccines containing both a DNA sequence encoding FIV gag protein and a DNA sequence encoding FIV env protein. The gag and env proteins are preferably expressed by baculovirus expression systems containing the DNA sequences encoding the FIV env and gag proteins or in feline herpes virus vectors containing the DNA sequences encoding the FIV env and gag proteins. Also disclosed are combined mucosal/parenteral, mucosal/mucosal and parenteral/parenteral inoculation methods.

30. 5,830,698, Nov. 3, 1998, Method for integrating genes at specific sites in mammalian cells via homologous recombination and vectors for accomplishing the same; Mitchell E. Reff, et al., 435/69.1, 320.1, 463, 465; 536/23.2 [IMAGE AVAILABLE]

US PAT NO: 5,830,698 [IMAGE AVAILABLE] L16: 30 of 100

ABSTRACT:
A method for achieving site specific integration of a desired DNA at a target site in a mammalian cell via homologous recombination is described. This method provides for the reproducible selection of cell lines wherein a desired DNA is integrated at a predetermined transcriptionally active site previously marked with a marker plasmid. The method is particularly suitable for the production of mammalian cell lines which secrete mammalian proteins at high levels, in particular immunoglobulins. Novel vectors and vector combinations for use in the subject cloning method are also provided.

31. 5,830,461, Nov. 3, 1998, Methods for promoting wound healing and treating transplant-associated vasculopathy; Timothy R. Billiar, et al., 424/94.4, 94.1; 435/189 [IMAGE AVAILABLE]

US PAT NO: 5,830,461 [IMAGE AVAILABLE] L16: 31 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 630,798, Apr. 10, 1996, which is a continuation-in-part of Ser. No. 265,046, Jun. 24, 1994, Pat. No. 5,658,565, and Ser. No. 465,522, Jun. 5, 1995, which is a division of Ser. No. 314,917, Sep. 28, 1994, Pat. No. 5,468,630, which is a continuation of Ser. No. 981,344, Nov. 25, 1992, abandoned.

ABSTRACT:
The present invention provides a method of promoting the closure of a wound in a patient. This method involves transferring exogenous iNOS to the region of the wound whereby a product of iNOS is produced in the region of the wound to promote the closure of the wound.

32. 5,817,492, Oct. 6, 1998, Recombinant DNA viral vector for transfecting animal cells; Izumu Saito, et al., 435/456; 424/93.21; 435/320.1, 465; 514/44 [IMAGE AVAILABLE]

US PAT NO: 5,817,492 [IMAGE AVAILABLE] L16: 32 of 100

ABSTRACT:
An animal cell is co-transfected with both a recombinant DNA viral vector which bears a **promoter**, a **recombinase** gene and a poly(A) sequence and a recombinant DNA viral vector which bears two **recombinase**-recognizing sequences and which further bears an origin of replication, a **promoter**, a foreign gene and a poly(A) sequence, each of which is located between the two **recombinase**-recognizing sequences. Thereafter, in the co-transfected animal cell, a DNA fragment containing the origin of replication, **promoter**, foreign gene and poly(A) sequence is excised from the vector by the action of a **recombinase** expressed in the another vector. The DNA fragment forms a circular DNA molecule which autonomously replicates in the co-transfected animal cell due to the origin of replication, whereby the foreign gene is continuously expressed. Accordingly, the combination of the above two DNA viral vectors wherein a gene which is defective in patients is used as the foreign gene is quite useful especially for the treatment of the patient with hereditary diseases.

33. 5,814,618, Sep. 29, 1998, Methods for regulating gene expression; Hermann Bujard, et al., 514/44; 424/93.21 [IMAGE AVAILABLE]

US PAT NO: 5,814,618 [IMAGE AVAILABLE] L16: 33 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 260,452, Jun. 14, 1994, Pat. No. 5,650,298, Ser. No. 76,726, Jun. 14, 1993, Pat. No. 5,464,758, Ser. No. 383,754, Feb. 6, 1995, and Ser. No. 275,876, Jul. 15, 1994, Pat. No. 5,654,168, which is a continuation-in-part of Ser. No. 270,637, Jul. 1, 1994, abandoned, said Ser. No. 260,452 is a continuation-in-part of Ser. No. 76,327, Jun. 14, 1993, abandoned.

ABSTRACT:
Methods of regulating gene expression in subjects using tetracycline-responsive fusion proteins are disclosed. In one embodiment, the method involves introducing into a cell the subject a nucleic acid molecule encoding a fusion protein which inhibits transcription, the fusion protein comprising a first polypeptide which binds to a tet operator sequence, operatively linked to a heterologous second polypeptide which inhibits transcription in eukaryotic cells; and modulating the concentration of a tetracycline, or analogue thereof, in the subject. The first polypeptide can binds to a tet operator sequence in the absence, but not the presence, of tetracycline. Alternatively, the first polypeptide can binds to a tet operator sequence in the presence, but not the absence, of tetracycline. In another embodiment, the method of the invention involves obtaining a cell from a subject, introducing into the cell a first nucleic acid molecule which operatively links a gene to at least one tet operator sequence, introducing into the cell a second nucleic acid molecule encoding an inhibitory fusion protein of the invention to form a modified cell, administering the modified cell to the subject and modulating the concentration of a tetracycline, or analogue thereof, in the subject. The first and second nucleic acid molecules can be linked or can be separate molecules.

34. 5,807,995, Sep. 15, 1998, Mammalian tumor susceptibility genes and their uses; Stanley N. Cohen, et al., 530/350 [IMAGE AVAILABLE]

US PAT NO: 5,807,995 [IMAGE AVAILABLE] L16: 34 of 100
REL-US-DATA: Division of Ser. No. 670,274, Jun. 13, 1996, which is a continuation-in-part of Ser. No. 585,758, Jan. 16, 1996, Pat. No. 5,679,523.

ABSTRACT:
TSG101 is a tumor susceptibility gene whose homozygous functional knock out in fibroblasts leads to transformation and the ability of these cells to form metastatic tumors in nude mice. The cellular transformation that results from inactivation of TSG101 is reversible by restoration of TSG101 function. Decreased expression of TSG101 is associated with the occurrence of certain human cancers, including breast carcinomas. The TSG101 nucleic acid compositions find use in identifying homologous or related proteins and the DNA sequences encoding such proteins; in producing compositions that modulate the expression or function of the protein; and in studying associated physiological pathways. In addition,

modulation of the gene activity in vivo is used for prophylactic and therapeutic purposes, such as treatment of cancer, identification of cell type based on expression, and the like. The DNA is further used as a diagnostic for a genetic predisposition to cancer, and to identify specific cancers having mutations in this gene.

35. 5,807,708, Sep. 15, 1998, Conservin nucleic acid molecules and compositions; Dean A. Falb, et al., 435/69.1, 252.3, 254.11, 320.1, 325; 536/23.1, 23.5 [IMAGE AVAILABLE]

US PAT NO: 5,807,708 [IMAGE AVAILABLE] L16: 35 of 100

ABSTRACT:
The present invention relates to the discovery of novel conservin genes and polypeptides. Therapeutics, diagnostics and screening assays based on these molecules are also disclosed.

36. 5,801,030, Sep. 1, 1998, Methods and vectors for site-specific recombination; Duncan L. McVey, et al., 435/456, 320.1, 462; 536/23.1, 23.2 [IMAGE AVAILABLE]

US PAT NO: 5,801,030 [IMAGE AVAILABLE] L16: 36 of 100

ABSTRACT:
The present invention provides methods for site-specific recombination in a cell, as well as vectors which can be employed in such methods. The methods and vectors of the present invention can be used to obtain persistent gene expression in a cell and to modulate gene expression. One preferred method according to the invention comprises contacting a cell with a vector comprising an origin of replication functional in mammalian cells located between first and second recombining sites located in parallel. Another preferred method comprises, in part, contacting a cell with a vector comprising first and second recombining sites in antiparallel orientations such that the vector is internalized by the cell. In both methods, the cell is further provided with a site-specific recombinase that effects recombination between the first and second recombining sites of the vector.

37. 5,800,998, Sep. 1, 1998, Assays for diagnosing type II diabetes in a subject; M. Alexandra Glucksmann, 435/6; 514/44; 536/23.1, 23.5 [IMAGE AVAILABLE]

US PAT NO: 5,800,998 [IMAGE AVAILABLE] L16: 37 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 748,229, Nov. 2, 1996, abandoned.

ABSTRACT:
Assays for determining whether a subject has or is at risk for developing type II diabetes, which are based on detecting the presence or absence of alterations in the hepatic nuclear factor 1 (HNF-1) gene or protein of the subject are disclosed.

38. 5,798,448, Aug. 25, 1998, AL-1 neurotrophic factor antibodies; Ingrid W. Caras, et al., 530/387.1; 424/9.34, 130.1, 132.1, 133.1, 134.1, 135.1, 136.1; 435/7.1, 7.2, 7.9; 436/512, 514, 517, 518, 536, 538, 547, 548; 530/300, 350, 387.3, 387.9, 388.1, 388.15, 388.24, 389.1 [IMAGE AVAILABLE]

US PAT NO: 5,798,448 [IMAGE AVAILABLE] L16: 38 of 100
REL-US-DATA: Continuation of Ser. No. 330,128, Oct. 27, 1994.

ABSTRACT:
The present invention provides nucleic acids encoding AL-1 protein, as well as AL-1 protein produced by recombinant DNA methods. Such AL-1 protein is useful in preparing antibodies and in diagnosing and treating various neuronal disorders.

39. 5,795,734, Aug. 18, 1998, EPH receptor ligands, and uses related thereto; John G. Flanagan, et al., 435/69.1, 7.1, 252.3, 320.1, 325; 530/300, 350; 536/23.1, 23.5 [IMAGE AVAILABLE]

US PAT NO: 5,795,734 [IMAGE AVAILABLE] L16: 39 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 393,462, Feb. 27, 1995, which is a continuation-in-part of Ser. No. 308,814, Sep. 19, 1994.

ABSTRACT:
The present invention relates to the discovery of a novel EPH receptor ligand, referred to hereinafter as "Elf-1", which protein has apparently broad involvement in the formation and maintenance of ordered spatial arrangements of differentiated tissues in vertebrates, and can be used to generate and/or maintain an array of different vertebrate tissue both in vitro and in vivo.

40. 5,795,726, Aug. 18, 1998, Methods for identifying compounds useful in treating type II diabetes; M. Alexandra Glucksmann, 435/7.21, 4, 6, 8; 536/23.5 [IMAGE AVAILABLE]

US PAT NO: 5,795,726 [IMAGE AVAILABLE] L16: 40 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 760,246, Dec. 4, 1996, which is a continuation-in-part of Ser. No. 749,431, Nov. 15, 1996, which is a continuation-in-part of Ser. No. 748,229, Nov. 12, 1996, abandoned.

ABSTRACT:
Methods for identifying compounds, which modulate the bioactivity of human hepatic nuclear factor-1 (HNF-1), and which are therefore useful in treating type II diabetes are disclosed.

41. 5,792,833, Aug. 11, 1998, E2 binding proteins; Elliot J. Androphy, et al., 530/350, 300 [IMAGE AVAILABLE]

US PAT NO: 5,792,833 [IMAGE AVAILABLE] L16: 41 of 100

ABSTRACT:
E2-BP polypeptides, nucleic acids encoding E2-BP polypeptides, and uses thereof.

42. 5,789,156, Aug. 4, 1998, Tetracycline-regulated transcriptional inhibitors; Hermann Bujard, et al., 435/6, 69.1, 69.7, 252.3, 320.1, 810; 536/23.4, 23.7, 24.1 [IMAGE AVAILABLE]

US PAT NO: 5,789,156 [IMAGE AVAILABLE] L16: 42 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 275,876, Jul. 15, 1994, Pat. No. 5,654,168, which is a continuation-in-part of Ser. No. 270,637, Jul. 1, 1994, abandoned, and a continuation-in-part of Ser. No. 260,452, Jun. 14, 1994, Pat. No. 5,650,298, which is a continuation-in-part of Ser. No. 76,327, Jun. 14, 1993, abandoned, and a continuation-in-part of Ser. No. 76,726, Jun. 14, 1993, Pat. No. 5,464,758.

ABSTRACT:
Nucleic acid molecules and proteins useful for regulating the expression of genes in eukaryotic cells and organisms in a highly controlled manner are disclosed. In the regulatory system of the invention, transcription of a tet operator-linked nucleotide sequence is inhibited by a transcriptional inhibitor fusion protein composed of two polypeptides, a first polypeptide which binds to tet operator sequences either (i) in the absence but not the presence of tetracycline (or an analogue thereof) or (ii) in the presence but not the absence of tetracycline (or an analogue thereof), and a second polypeptide which directly or indirectly inhibits transcription in eukaryotic cells. In one embodiment, the fusion protein comprises a Tet repressor operatively linked to a transcriptional silencer polypeptide. In another embodiment, the fusion protein comprises a mutated Tet repressor operatively linked to a transcriptional silencer polypeptide. The fusion proteins of the invention are useful for reducing the level of transcription of a tet operator-linked target gene. Moreover, the fusion proteins of the invention can be used in combination with tetracycline-regulated transcriptional activator fusion proteins to allow for precise regulation of the expression of one or multiple target genes. Kits including the components of the regulatory system of the invention are also encompassed by the invention.

43. 5,780,296, Jul. 14, 1998, Compositions and methods to promote homologous recombination in eukaryotic cells and organisms; William K. Holloman, et al., 435/320.1; 536/23.2 [IMAGE AVAILABLE]

US PAT NO: 5,780,296 [IMAGE AVAILABLE] L16: 43 of 100

ABSTRACT:
The invention concerns genes encoding recombinases that can be used to promote homologous recombination in eukaryotic cells and expression vectors that can be used to transiently express recombinases in target cells. One embodiment of the invention encompasses genetically engineered nucleic acids that encode a non-naturally occurring recombinase that causes a greater rate of recombination than does the naturally occurring recombinase. Recombinases from Ustilago maydis, Saccharomyces cerevisiae

are specifically included in the invention.

44. 5,776,449, Jul. 7, 1998, Recombinant bacillus thuringiensis strains, insecticidal compositions and method of use; James A. Baum, 424/93.2, 93.461, 405; 435/170, 252.31, 832 [IMAGE AVAILABLE]

US PAT NO: 5,776,449 [IMAGE AVAILABLE] L16: 44 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 266,408, Jun. 24, 1994, which is a continuation-in-part of Ser. No. 89,986, Jul. 8, 1993, Pat. No. 5,441,884.

ABSTRACT:
A transposable element, or transposon, isolated from Bacillus thuringiensis (B.t.) and designated as transposon Tn5401. The invention also includes a method of using this transposon in a site-specific recombination system for construction of recombinant B.t. strains that contain insecticidal B.t. toxin protein genes and that are free of DNA not native to B.t., insecticidal compositions containing recombinant B.t. strains and their use in insect control methods.

45. 5,773,697, Jun. 30, 1998, Genetic constructs and methods for producing fruits with very little or diminished seed; Dwight T. Tomes, et al., 800/260; 435/69.1, 320.1; 536/23.7, 24.1; 800/268, 287, 290, 298, 308, 309 [IMAGE AVAILABLE]

US PAT NO: 5,773,697 [IMAGE AVAILABLE] L16: 45 of 100

ABSTRACT:
The invention discloses a transgenic method for producing fruit with diminished or very little seeds or fruits with reduced seed number. It involves the temporal expression of a cytotoxic gene or combination of genes targeted toward arresting seed development at a time sufficiently after pollination that fruit development and maturation is normal while early enough in seed development that seed maturation per se is minimized. The invention also includes transgenic constructs, vectors, and methods for production of the fruit with diminished or very little seed bearing plants.

46. 5,770,384, Jun. 23, 1998, Method for determining compound interaction with E2 binding proteins; Elliot J. Androphy, et al., 435/7.8, 5, 7.1, 7.93, 69.1, 69.7; 514/2; 530/300, 350; 536/23.72 [IMAGE AVAILABLE]

US PAT NO: 5,770,384 [IMAGE AVAILABLE] L16: 46 of 100
REL-US-DATA: Division of Ser. No. 361,806, Dec. 22, 1994.

ABSTRACT:
E2-BP polypeptides, nucleic acids encoding E2-BP polypeptides, and uses thereof.

47. 5,763,240, Jun. 9, 1998, In vivo homologous sequence targeting in eukaryotic cells; David A. Zarling, et al., 435/463, 6, 91.1, 91.4 [IMAGE AVAILABLE]

US PAT NO: 5,763,240 [IMAGE AVAILABLE] L16: 47 of 100
REL-US-DATA: Continuation of Ser. No. 873,438, Apr. 24, 1992, abandoned.

ABSTRACT:
The invention relates to methods for targeting an exogenous polynucleotide or exogenous complementary polynucleotide pair to a predetermined endogenous DNA target sequence in a eukaryotic cell by homologous pairing, particularly for altering an endogenous DNA sequence, such as a chromosomal DNA sequence, typically by targeted homologous recombination. In certain embodiments, the invention relates to methods for targeting an exogenous polynucleotide having a linked chemical substituent to a predetermined endogenous DNA sequence in a metabolically active eukaryotic cell, generating a DNA sequence-specific targeting of one or more chemical substituents in an intact nucleus of a metabolically active eukaryotic cell, generally for purposes of altering a predetermined endogenous DNA sequence in the cell. The invention also relates to compositions that contain exogenous targeting polynucleotides, complementary pairs of exogenous targeting polynucleotides, chemical substituents of such polynucleotides, and recombinase proteins used in the methods of the invention.

48. 5,759,775, Jun. 2, 1998, Methods for detecting nucleic acids encoding AL-1 neurotrophic factor; Ingrid W. Caras, et al., 435/6, 91.2; 536/23.5, 24.31, 24.33 [IMAGE AVAILABLE]

US PAT NO: 5,759,775 [IMAGE AVAILABLE] L16: 48 of 100
REL-US-DATA: Continuation of Ser. No. 330,128, Oct. 27, 1994.

ABSTRACT:
Provided are nucleic acids encoding AL-1 protein, as well as AL-1 protein produced by recombinant DNA methods. Such AL-1 protein is useful in preparing antibodies and in diagnosing and treating various neuronal disorders. The present invention provides methods to preferentially detect or amplify AL-1 nucleic acid in a sample using AL-1 nucleotide sequence probes.

49. 5,756,671, May 26, 1998, CDC37 cell-cycle regulatory protein, and uses related thereto; Jeno Gyuris, et al., 530/350, 300 [IMAGE AVAILABLE]

US PAT NO: 5,756,671 [IMAGE AVAILABLE] L16: 49 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 466,679, Jun. 6, 1995, abandoned, which is a continuation-in-part of Ser. No. 253,155, Jun. 2, 1994, Pat. No. 5,691,147.

ABSTRACT:
The present invention relates to the discovery in mammalian cells, particularly human cells, of a novel CDK-binding protein, referred to herein as "cdc37". As described herein, this protein functions to facilitate activation and accordingly functions in the modulation of cell-cycle progression, and therefore ultimately of cell growth and differentiation. Moreover, binding data indicated that cdc37 may function coordinately with other cell-cycle regulatory proteins, such as of cyclin-dependent kinases (CDKs), src, p53 and erk kinases.

50. 5,747,035, May 5, 1998, Polypeptides with increased half-life for use in treating disorders involving the LFA-1 receptor; Leonard G. Presta, et al., 424/144.1, 130.1, 133.1, 135.1, 141.1, 143.1, 153.1, 154.1, 173.1; 514/2, 8, 885; 530/387.1 [IMAGE AVAILABLE]

US PAT NO: 5,747,035 [IMAGE AVAILABLE] L16: 50 of 100

ABSTRACT:
Polypeptides that are cleared from the kidney and do not contain in their original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding epitope of an Fc region of an IgG and thereby have increased circulatory half-life. Methods are described herein which utilize these polypeptides in treating disorders involving the LFA-1 receptor. In one of the described methods of treatment, the polypeptide includes the amino acid sequence PKNSSMISNTP (SEQ ID NO:3) and may also
include the sequence selected from the group consisting of HQNLSDGK (SEQ ID NO: 1), HQNISDGK (SEQ ID NO:2), HQSLGTQ (SEQ ID NO:11) and VISSHLGQ
(SEQ ID NO:31).

51. 5,744,343, Apr. 28, 1998, Ubiquitin conjugating enzymes; Giulio Draetta, et al., 435/193, 252.3, 254.11, 320.1, 325; 536/23.2, 23.4 [IMAGE AVAILABLE]

US PAT NO: 5,744,343 [IMAGE AVAILABLE] L16: 51 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 247,904, May 23, 1994, which is a continuation-in-part of Ser. No. 176,937, Jan. 4, 1994, abandoned.

ABSTRACT:
The present invention concerns three ubiquitin-conjugating enzymes.

52. 5,744,336, Apr. 28, 1998, DNA constructs for controlled transformation of eukaryotic cells; Thomas K. Hodges, et al., 435/320.1; 536/23.1, 24.1, 24.2 [IMAGE AVAILABLE]

US PAT NO: 5,744,336 [IMAGE AVAILABLE] L16: 52 of 100
REL-US-DATA: Division of Ser. No. 10,997, Jan. 29, 1993, Pat. No. 5,527,695.

ABSTRACT:
DNA constructs are provided for the creation of transgenic eukaryotic cells. These DNA constructs allow a more precise and effective transformation procedure by enabling the targeting of DNA sequences for insertion into a particular DNA locus, while enabling the removal of any randomly inserted DNA sequences that occur as a by product of known transformation procedures.

53. 5,739,277, Apr. 14, 1998, Altered polypeptides with increased half-life; Leonard G. Presta, et al., 530/326, 300, 350, 387.1 [IMAGE AVAILABLE]

US PAT NO: 5,739,277 [IMAGE AVAILABLE] L16: 53 of 100

ABSTRACT:
Polypeptides that are cleared from the kidney and do not contain in their original form a Fc region of an IgG are altered so as to comprise a salvage receptor binding epitope of an Fc region of an IgG and thereby have increased circulatory half-life.

54. 5,736,377, Apr. 7, 1998, NES-1 polypeptides, DNA, and related molecules and methods; Vimla Band, 435/219, 212, 226; 530/350 [IMAGE AVAILABLE]

US PAT NO: 5,736,377 [IMAGE AVAILABLE] L16: 54 of 100

ABSTRACT:
The expression and purification of normal epithelial specific polypeptide (NES1) which is expressed in normal cells, but not in radiation transformed cells, are described. Both the DNA sequence encoding the NES1 and the corresponding amino acid sequence are disclosed. Also, disclosed are methods for carcinoma detection and treatment using the NES1 as well as methods of identifying compounds modulating the expression and activity of NES1.

55. 5,733,761, Mar. 31, 1998, Protein production and protein delivery; Douglas Treco, et al., 435/463, 69.4; 536/23.51, 24.1 [IMAGE AVAILABLE]

US PAT NO: 5,733,761 [IMAGE AVAILABLE] L16: 55 of 100
REL-US-DATA: Continuation of Ser. No. 985,586, Dec. 3, 1992, abandoned, which is a continuation-in-part of Ser. No. 789,188, Nov. 5, 1991, abandoned, Ser. No. 911,533, Jul. 10, 1992, abandoned, and Ser. No. 787,840, Nov. 5, 1991, abandoned.

ABSTRACT:
The present invention relates to transfected primary, secondary and immortalized cells of vertebrate origin, particularly mammalian origin, transfected with exogenous genetic material (DNA) which encodes a desired (e.g., a therapeutic) product or is itself a desired (e.g., therapeutic) product, methods by which primary, secondary and immortalized cells are transfected to include exogenous genetic material, including DNA targeting by homologous recombination, methods for the activation and amplification of endogenous cellular genes, methods by which cells useful for large-scale protein production can be obtained, methods of producing clonal cell strains or heterogenous cell strains, and methods of gene therapy in which transfected primary, secondary or immortalized cells are used. The present invention includes primary, secondary and immortalized cells, such as fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, formed elements of the blood, muscle cells, and other cells which can be cultured.

56. 5,733,743, Mar. 31, 1998, Methods for producing members of specific binding pairs; Kevin Stuart Johnson, et al., 435/69.1, 252.33, 320.1, 462, 472; 530/387.1 [IMAGE AVAILABLE]

US PAT NO: 5,733,743 [IMAGE AVAILABLE] L16: 56 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 150,002, Nov. 12, 1993.

ABSTRACT:

Methods, recombinant host cells and kits are disclosed for the production of members of specific binding pairs (sbp), e.g. antibodies, using display on the surface of secreted replicable genetic display packages (rgdps), e.g. filamentous phage. To produce a library of great diversity, recombination occurs between first and second vectors comprising nucleic acid encoding first and second polypeptide chains of sbp members respectively, thereby producing recombinant vectors each encoding both a first and a second polypeptide chain component of an sbp member. The recombination may take place in vitro or intracellularly and may be site-specific, e.g. involving use of the loxP sequence and mutants thereof. Recombination may take place after prior screening or selecting for rgdps displaying sbp members which bind complementary sbp member of interest.

57. 5,731,182, Mar. 24, 1998, Non-mammalian DNA virus to express an exogenous gene in a mammalian cell; Frederick M. Boyce, 435/183, 69.1, 70.1, 320.1 [IMAGE AVAILABLE]

US PAT NO: 5,731,182 [IMAGE AVAILABLE] L16: 57 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 311,157, Sep. 23, 1994.

ABSTRACT:
Disclosed is a method of expressing an exogenous gene in a mammalian cell, involving infecting the cell with a non-mammalian virus (e.g., a baculovirus) whose genome carries an exogenous gene, and growing the cell under conditions such that the gene is expressed. Also disclosed is a method of treating a gene deficiency disorder in a mammal by providing to a cell a therapeutically effective amount of a virus whose genome carries an exogenous gene and growing the cell under conditions such that the exogenous gene is expressed in the mammal.

58. 5,723,765, Mar. 3, 1998, Control of plant gene expression; Melvin John Oliver, et al., 800/268; 435/320.1, 418, 419; 536/23.6, 24.1, 24.5; 800/287, 288, 314 [IMAGE AVAILABLE]

US PAT NO: 5,723,765 [IMAGE AVAILABLE] L16: 58 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 283,604, Aug. 1, 1994, abandoned.

ABSTRACT:
A method for making a genetically modified plant comprising regenerating a whole plant from a plant cell that has been transfected with DNA sequences comprising a first gene whose expression results in an altered plant phenotype linked to a transiently active **promoter**, the gene and **promoter** being separated by a blocking sequence flanked on either side by specific excision sequences, a second gene that encodes a **recombinase** specific for the specific excision sequences linked to a repressible **promoter**, and a third gene that encodes the repressor specific for the repressible **promoter**. Also a method for making a genetically modified hybrid plant by hybridizing a first plant regenerated from a plant cell that has been transfected with DNA sequences comprising a first gene whose expression results in an altered plant phenotype linked to a transiently active **promoter**, the gene and **promoter** being separated by a blocking sequence flanked on either side by specific excision sequences to a second plant regenerated from a second plant cell that has been transfected with DNA sequences comprising a second gene that encodes a **recombinase** specific for the specific excision sequences linked to a **promoter** that is active during seed germination, and growing a hybrid plant from the hybrid seed. Plant cells, plant tissues, plant seed and whole plants containing the above DNA sequences are also claimed.

59. 5,723,585, Mar. 3, 1998, Method of purifying cardiac hypertrophy factor; Joffre Baker, et al., 530/413, 350, 380; 930/140 [IMAGE AVAILABLE]

US PAT NO: 5,723,585 [IMAGE AVAILABLE] L16: 59 of 100
REL-US-DATA: Division of Ser. No. 286,304, Aug. 5, 1994, Pat. No. 5,571,893, which is a continuation-in-part of Ser. No. 233,609, Apr. 25, 1994, Pat. No. 5,534,615.

ABSTRACT:
Isolated CHF, isolated DNA encoding CHF, recombinant or synthetic methods of preparing CHF, and a method of purifying CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

60. 5,723,333, Mar. 3, 1998, Human pancreatic cell lines: developments and uses; Fred Levine, et al., 435/325, 320.1, 377, 378 [IMAGE AVAILABLE]

US PAT NO: 5,723,333 [IMAGE AVAILABLE] L16: 60 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 386,897, Feb. 10, 1995, abandoned.

ABSTRACT:
This invention relates to cell lines, particularly mammalian cell lines, established by transforming the cells with vectors, preferably retroviral vectors, containing two or more oncogenes under the control of one or more inducible promoters and/or genetic elements. Also within the scope of the invention are human cell lines with extended in vitro lifespan, transformed by vectors containing one or more oncogenes under the control of one or more, preferably exogenous, inducible promoters and/or genetic

elements. The vectors may additionally contain gene(s) encoding for desired gene product(s). Also disclosed are insulin producing human pancreatic cell lines useful for transplantation into human diabetic patients.

61. 5,721,118, Feb. 24, 1998, Mammalian artificial chromosomes and methods of using same; Immo E. Scheffler, 435/69.1, 320.1, 325, 449; 514/44; 536/23.1, 23.5 [IMAGE AVAILABLE]

US PAT NO: 5,721,118 [IMAGE AVAILABLE] L16: 61 of 100

ABSTRACT:
The present invention provides a mammalian artificial chromosome (MAC), comprising a centromere and a unique cloning site, said MAC containing less than 0.1% of the DNA present in a normal haploid genome of the mammalian cell from which the centromere was obtained. The invention further provides a MAC, wherein the unique cloning site is a nucleic acid sequence encoding a selectable marker. The invention also provides methods of preparing a MAC. In addition, the invention provides methods of stably expressing a selectable marker in a cell, comprising introducing a MAC containing the selectable marker into the cell. The invention also provides a cell containing a MAC expressing an exogenous nucleic acid sequence and a transgenic mammal expressing a selectable marker.

62. 5,709,858, Jan. 20, 1998, Antibodies specific for Rse receptor protein tyrosine kinase; Paul J. Godowski, et al., 424/143.1, 139.1; 435/7.4; 530/387.3, 387.9, 388.22, 391.1, 391.3 [IMAGE AVAILABLE]

US PAT NO: 5,709,858 [IMAGE AVAILABLE] L16: 62 of 100
REL-US-DATA: Continuation of Ser. No. 170,558, Dec. 20, 1993, which is a continuation of Ser. No. 157,563, Nov. 23, 1993, abandoned.

ABSTRACT:
The protein tyrosine kinase receptors, designated Rse and HPTK6, have been purified from human and/or murine cell tissues. Rse and HPTK6 have been cloned from a cDNA library of a human liver carcinoma cell line (i.e., Hep 3B) using PCR amplification. Provided herein are nucleic acid sequences encoding Rse and HPTK6 useful as diagnostics and in the recombinant preparation of Rse and HPTK6. Rse and HPTK6 are used in the preparation and purification of antibodies thereto and in diagnostic assays.

63. 5,700,470, Dec. 23, 1997, Recombinant adenovirus with removed EZA gene and method of preparation; Izumu Saito, et al., 424/233.1, 199.1; 435/252.32, 320.1, 456; 514/44; 536/23.72 [IMAGE AVAILABLE]

US PAT NO: 5,700,470 [IMAGE AVAILABLE] L16: 63 of 100

ABSTRACT:
A recombinant DNA virus for transfecting an animal cell and bearing a foreign gene and a promoter capable of regulating expression of the foreign gene is completely deleted of the function of E2A gene. The recombinant DNA virus can thus stably transduce the foreign gene into various animal cells, which leads to continuous expression of the foreign gene in the animal cells. The continuous expression of the foreign gene can provide an effective treatment of hereditary disease.

64. 5,695,977, Dec. 9, 1997, Site directed recombination; Jerzy W. Jurka, 435/463; 536/23.1, 24.5 [IMAGE AVAILABLE]

US PAT NO: 5,695,977 [IMAGE AVAILABLE] L16: 64 of 100

ABSTRACT:
Enhanced homologous recombination is obtained by employing a consensus sequence which has been found to be associated with integration of repeat sequences, such as Alu and ID. The consensus sequence or sequence having a single transition mutation determines one site of a double break which allows for high efficiency of integration at the site. By introducing single or double stranded DNA having the consensus sequence flanking region joined to a sequence of interest, one can reproducibly direct integration of the sequence of interest at one or a limited number of sites. In this way, specific sites can be identified and homologous recombination achieved at the site by employing a second flanking sequence associated with a sequence proximal to the 3'-nick.

65. 5,679,545, Oct. 21, 1997, Gene encoding cardiac hypertrophy factor; Joffre Baker, et al., 435/69.1, 252.3, 320.1, 325; 536/23.5 [IMAGE AVAILABLE]

US PAT NO: 5,679,545 [IMAGE AVAILABLE] L16: 65 of 100
REL-US-DATA: Division of Ser. No. 286,304, Aug. 5, 1994, Pat. No. 5,571,893, Nov. 5, 1996, which is a continuation-in-part of Ser. No. 233,609, Apr. 25, 1994, Pat. No. 5,534,615, Jul. 9, 1996.

ABSTRACT:
Isolated CT-1, isolated DNA encoding CT-1, and recombinant or synthetic methods of preparing CT-1 are disclosed. These CT-1 molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

66. 5,679,523, Oct. 21, 1997, Method for concurrent disruption of expression of multiple alleles of mammalian genes; Limin Li, et al., 435/6, 320.1, 463; 536/23.2, 24.5 [IMAGE AVAILABLE]

US PAT NO: 5,679,523 [IMAGE AVAILABLE] L16: 66 of 100

ABSTRACT:
Methods are provided for identifying a gene at a random chromosomal locus in the genome of a mammalian cell. The method involves inactivating one copy of the gene by integrating one DNA construct (knockout construct) in that gene copy. The knockout construct includes a positive selection marker region sequence and, in a 5' direction from the selection marker region sequence, a transcription initiation region sequence responsive to a transactivation factor, said transcription initiation region oriented for antisense RNA transcription in the direction away from the selection marker region sequence. The second copy of the gene is inactivated by transforming the cells with a second DNA construct (transactivation construct) containing a gene sequence for the transactivation factor which initiates antisense RNA transcription extending from the knockout construct into the chromosomal locus flanking the knockout construct at its 5' end. Inactivation of both gene copies may result in a change in cell phenotype distinguishable from the wild-type phenotype. Optionally, the wild-type phenotype can be regained by introducing a third construct that can inhibit antisense RNA transcription.

67. 5,659,022, Aug. 19, 1997, Oligonucleotide-cyclopropapyrroloindole conjugates as sequence specific hybridization and crosslinking agents for nucleic acids; Igor V. Kutyavin, et al., 536/22.1, 23.1, 24.3 [IMAGE AVAILABLE]

US PAT NO: 5,659,022 [IMAGE AVAILABLE] L16: 67 of 100

ABSTRACT:
Covalently linked conjugates of oligonucleotides (ODNs) with a cyclopropapyrroloindole moiety or an analog thereof, selectively and efficiently alkylate and crosslink with nucleic acid sequences that are complementary to the base sequence of the ODN.

68. 5,656,438, Aug. 12, 1997, CAIP-like gene family; Yen-Ming Hsu, 435/7.1, 7.2; 530/387.9, 388.73, 388.75 [IMAGE AVAILABLE]

US PAT NO: 5,656,438 [IMAGE AVAILABLE] L16: 68 of 100

ABSTRACT:
CAIP polypeptide, nucleic acids, antibodies thereto and uses thereof.

69. 5,654,168, Aug. 5, 1997, Tetracycline-inducible transcriptional activator and tetracycline-regulated transcription units; Hermann Bujard, et al., 435/69.1, 320.1; 536/23.7, 24.1 [IMAGE AVAILABLE]

US PAT NO: 5,654,168 [IMAGE AVAILABLE] L16: 69 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 270,637, Jul. 1, 1994, abandoned.

ABSTRACT:
Nucleic acid molecules and proteins useful for regulating the expression of genes in eukaryotic cells and organisms in an inducible manner are disclosed. In the regulatory system of the invention, transcription of a tet operator-linked nucleotide sequence is stimulated by a transcriptional activator fusion protein composed of two polypeptides, a first polypeptide which binds to tet operator sequences in the presence of tetracycline or a tetracycline analogue and a second polypeptide which directly or indirectly activates transcription in eukaryotic cells. In

one embodiment, the fusion protein comprises a mutated Tet repressor operatively linked to a transcriptional activation polypeptide, such as a portion of herpes simplex virus virion protein 16. In the absence of an inducing agent (tetracycline or a tetracycline analogue), transcription of the tet operator-linked nucleotide sequence remains uninduced. In the presence of the inducing agent, transcription of the tet operator-linked nucleotide sequence is stimulated by the transactivator fusion protein of the invention. Novel transcription units which allow for coordinate or independent tetracycline-regulated expression of two or more nucleotide sequences by the transactivator fusion protein of the invention are also disclosed. Kits including the components of the regulatory system of the invention are also encompassed by the invention.

70. 5,650,308, Jul. 22, 1997, Recombinant Bacillus thuringiensis strain construction method; James A. Baum, 435/485, 252.31, 320.1 [IMAGE AVAILABLE]

US PAT NO: 5,650,308 [IMAGE AVAILABLE] L16: 70 of 100
REL-US-DATA: Division of Ser. No. 89,986, Jul. 8, 1993, Pat. No. 5,441,884.

ABSTRACT:
A transposable element, or transposon, isolated from Bacillus thuringiensis (B.t.) and designated as transposon Tn5401. The invention also includes a method of using this transposon in a site-specific recombination system for construction of recombinant B.t. strains that contain insecticidal B.t. toxin protein genes and that are free of DNA not native to B.t.

71. 5,650,298, Jul. 22, 1997, Tight control of gene expression in eucaryotic cells by tetracycline-responsive promoters; Hermann Bujard, et al., 435/69.7, 320.1, 463; 536/23.4, 24.1 [IMAGE AVAILABLE]

US PAT NO: 5,650,298 [IMAGE AVAILABLE] L16: 71 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 76,327, Jun. 14, 1993, abandoned.

ABSTRACT:
Transgenic animals carrying two transgenes, the first coding for a transactivator fusion protein comprising a tet repressor and a polypeptide which directly or indirectly activates in eucaryotic cells, and the second comprising a gene operably linked to a minimal promoter operably linked to at least one tet operator sequence, are disclosed. Isolated DNA molecules (e.g., targeting vectors) for integrating a polynucleotide sequence encoding a transactivator of the invention at a predetermined location within a second target DNA molecule by homologous recombination are also disclosed. Transgenic animals having the DNA molecules of the invention integrated at a predetermined location in a chromosome by homologous recombination are also encompassed by the invention. Methods to regulate the expression of a tet operator linked-gene of interest by administering tetracycline or a tetracycline analogue to an animal of the invention are also disclosed. The regulatory system of the invention allows for conditional inactivation or modulation of expression of a gene of interest in a host cell or animal.

72. 5,641,748, Jun. 24, 1997, Caip-like gene family; Yen-Ming Hsu, 514/12; 435/7.8; 530/324, 350 [IMAGE AVAILABLE]

US PAT NO: 5,641,748 [IMAGE AVAILABLE] L16: 72 of 100

ABSTRACT:
A substantially pure preparation of a polypeptide, the sequence of which comprises the sequence of a CAIP polypeptide.

73. 5,641,670, Jun. 24, 1997, Protein production and protein delivery; Douglas A. Treco, et al., 1/1; 435/254.11, 320.1, 326, 366, 367, 371, 372, 372.2, 372.3 [IMAGE AVAILABLE]

US PAT NO: 5,641,670 [IMAGE AVAILABLE] L16: 73 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 985,586, Dec. 3, 1992, abandoned, which is a continuation-in-part of Ser. No. 789,188, Nov. 5, 1991, abandoned, Ser. No. 911,533, Jul. 10, 1992, abandoned, and Ser. No. 787,840, Nov. 5, 1991, abandoned.

ABSTRACT:
The invention relates to constructs comprising: a) a targeting sequence; b) a regulatory sequence; c) an exon; and d) an unpaired splice-donor site. The invention further relates to a method of producing protein in vitro or in vivo comprising the homologous recombination of a construct as described above within a cell. The homologously recombinant cell is then maintained under conditions which will permit transcription and translation, resulting in protein expression. The present invention further relates to homologously recombinant cells, including primary, secondary, or immortalized vertebrate cells, methods of making the cells, methods of homologous recombination to produce fusion genes, methods of altering gene expression in the cells, and methods of making a protein in a cell employing the constructs of the invention.

74. 5,629,159, May 13, 1997, Immortalization and disimmortalization of cells; David J. Anderson, 435/6, 69.1, 194, 325, 357, 363, 366, 368, 372, 462, 467 [IMAGE AVAILABLE]

US PAT NO: 5,629,159 [IMAGE AVAILABLE] L16: 74 of 100

ABSTRACT:
Methods and compositions for the conditional immortalization of cells are provided.

75. 5,627,073, May 6, 1997, Hybridomas producing antibodies to cardiac hypertrophy factor; Joffre Baker, et al., 435/331; 424/139.1, 145.1; 435/69.6, 70.21, 252.33, 332, 336; 530/387.3, 387.9, 388.23, 391.3 [IMAGE AVAILABLE]

US PAT NO: 5,627,073 [IMAGE AVAILABLE] L16: 75 of 100
REL-US-DATA: Division of Ser. No. 286,304, Aug. 5, 1994, which is a continuation-in-part of Ser. No. 233,609, Apr. 25, 1994, abandoned.

ABSTRACT:
Isolated CHF (also referred to cardiac hypertrophy factor or cardiotrophin-1), isolated DNA encoding CHF, hybridomas and cell lines producing antibodies to CHF, and recombinant or synthetic methods of preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

76. 5,624,899, Apr. 29, 1997, Method for using Htk ligand; Brian D. Bennett, et al., 514/12, 2; 530/350 [IMAGE AVAILABLE]

US PAT NO: 5,624,899 [IMAGE AVAILABLE] L16: 76 of 100
REL-US-DATA: Division of Ser. No. 277,722, Jul. 20, 1994.

ABSTRACT:
A novel hepatoma transmembrane kinase receptor ligand (Htk ligand) which binds to, and activates, the Htk receptor is disclosed. As examples, mouse and human Htk ligands have been identified in a variety of tissues using a soluble Htk-Fc fusion protein. The ligands have been cloned and sequenced. The invention also relates to nucleic acids encoding the ligand, methods for production and use of the ligand, and antibodies directed thereto.

77. 5,624,806, Apr. 29, 1997, Antibodies to cardiac hypertrophy factor and uses thereof; Joffre Baker, et al., 435/7.1, 331, 344.1; 530/387.3, 387.9, 388.85, 391.3 [IMAGE AVAILABLE]

US PAT NO: 5,624,806 [IMAGE AVAILABLE] L16: 77 of 100
REL-US-DATA: Division of Ser. No. 286,304, Aug. 5, 1994, which is a continuation of Ser. No. 233,609, Apr. 25, 1994, Pat. No. 5,534,615.

ABSTRACT:
Isolated CHF, isolated DNA encoding cardiac hypertrophy factor (CHF), and recombinant or synthetic methods of preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

78. 5,622,856, Apr. 22, 1997, High efficiency helper system for AAV vector production; Georges Natsoulis, 435/325, 69.1, 320.1, 348, 366, 367, 369; 536/23.72 [IMAGE AVAILABLE]

US PAT NO: 5,622,856 [IMAGE AVAILABLE] L16: 78 of 100

ABSTRACT:

Novel nucleic acid molecules are provided having adeno-associated virus (AAV) coding regions that are capable of expressing necessary AAV functions to complement an AAV vector in the production of recombinant AAV (rAAV) virions. The molecules feature a nucleotide sequence that is substantially homologous to an AAV p5 promoter region, wherein the p5 promoter region is situated in the molecules in a site that is other than its natural position relative to the AAV rep coding region in the wild-type AAV genome. AAV helper function constructs are also provided, comprising the instant nucleic acid molecules embodied in a replicon that is capable of being transcribed and translated to express complementing AAV helper functions in a suitable host cell. Novel AAV packaging cells and AAV producer cells are provided, which contain the AAV helper constructs of the invention, and methods of producing enhanced levels of rAAV virions using the AAV helper constructs of the invention are also provided. Methods are also provided for producing rAAV virions without the concomitant production of significant levels of wild-type AAV.

79. 5,596,089, Jan. 21, 1997, Oligonucleotide probe and primers specific to bovine or porcine male genomic DNA; David W. Silversides, et al., 536/24.3; 435/6, 91.2, 810; 436/501; 536/23.1, 24.1, 24.31, 24.32, 24.33 [IMAGE AVAILABLE]

US PAT NO: 5,596,089 [IMAGE AVAILABLE] L16: 79 of 100

ABSTRACT:
The present invention relates to novel bovine (SEQ ID NO:1) and porcine (SEQ ID NO:2) genomic sequences for the SRY gene along with oligonucleotide primers. The present invention also relates to a method of sexing bovine or porcine tissue by descriminating PCR products obtained by amplification of specific DNA or cDNA sequences of bovine or porcine tissue which is used as a DNA template and wherein two pairs of DNA primers are used for the PCR. The present invention also relates to a method for the genetic manipulation or selection of sexual phenotype in domesticated animals, which comprises using transgenes composed of SRY sequences to cause and control the expression of genetic ablation sequences and genetic switching sequences in undifferentiated and developing gonadal tissues of both XX and XY animals.

80. 5,589,362, Dec. 31, 1996, Tetracycline regulated transcriptional modulators with altered DNA binding specificities; Hermann Bujard, et al., 435/69.1, 320.1, 325, 358, 455; 536/23.4, 24.1 [IMAGE AVAILABLE]

US PAT NO: 5,589,362 [IMAGE AVAILABLE] L16: 80 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 383,754, Feb. 3, 1995, and a continuation-in-part of Ser. No. 275,876, Jul. 15, 1994, and a continuation-in-part of Ser. No. 260,452, Jun. 14, 1994, and a continuation-in-part of Ser. No. 76,726, Jun. 14, 1993, Pat. No. 5,464,758, said Ser. No. 275,876 is a continuation-in-part of Ser. No. 270,637, Jul. 1, 1994, abandoned, said Ser. No. 260,452 is a continuation-in-part of Ser. No. 76,327, Jun. 14, 1993, abandoned.

ABSTRACT:
Isolated nucleic acid molecules encoding fusion proteins which regulate transcription in eukaryotic cells are disclosed. The fusion proteins of the invention comprise a Tet repressor having at least one amino acid mutation that confers on the fusion protein an ability to bind a class B tet operator sequence having a nucleotide substitution at position +4 or +6, operatively linked to a polypeptide which regulates transcription in eukaryotic cells. Methods for regulating transcription of a tet operator-linked gene in a cell are also provided. In one embodiment, the method involves introducing into the cell a nucleic acid molecule encoding a fusion protein which regulates transcription, the fusion protein comprising a Tet repressor having at least one amino acid mutation that confers on the fusion protein an ability to bind a class B tet operator sequence having a nucleotide substitution at position +4 or +6, operatively linked to a polypeptide which regulates transcription in eukaryotic cells, and modulating the concentration of a tetracycline, or analogue thereof, in contact with the cell.

81. 5,583,278, Dec. 10, 1996, Recombination activating gene deficient mouse; Frederick W. Alt, et al., 800/11; 424/9.2, 204.1, 234.1; 435/320.1; 800/18, 24 [IMAGE AVAILABLE]

US PAT NO: 5,583,278 [IMAGE AVAILABLE] L16: 81 of 100
REL-US-DATA: Continuation of Ser. No. 847,565, Mar. 5, 1992, abandoned.

ABSTRACT:
This invention relates to a recombinant mouse with both alleles of recombination activating gene 2 being functionally deficient. This invention discloses the method to make such mouse and the uses of such mouse.

82. 5,578,461, Nov. 26, 1996, Gene manipulation and expression using genomic elements; Stephen Sherwin, et al., 435/69.1, 244, 320.1, 464; 536/23.1, 24.1 [IMAGE AVAILABLE]

US PAT NO: 5,578,461 [IMAGE AVAILABLE] L16: 82 of 100
REL-US-DATA: Continuation of Ser. No. 1,898, Jan. 7, 1993, abandoned, which is a continuation-in-part of Ser. No. 432,069, Nov. 6, 1989, abandoned.

ABSTRACT:
Expression of mammalian target genes is achieved by employing chromosomal target DNA, either native primary cells or YACs in a yeast host, where the YACs include a fragment of a mammalian chromosome, the fragment comprising the target gene. Employing homologous recombination, an amplifiable gene is integrated into the mammalian fragment at a site to allow for amplification. In the same step, or one or more steps, as desired, the mammalian gene and/or the transcriptional system may be modified by in vivo mutagenesis. The resulting construct from homologous recombination may then be transformed into a mammalian expression host and integrated into the host genome, either randomly or by homologous recombination. The amplifiable gene may then be amplified by an appropriate agent providing for multiple copies of the target gene and the expression host grown to provide for high yields of the desired wild-type or modified protein.

83. 5,571,893, Nov. 5, 1996, Cardiac hypertrophy factor; Joffre Baker, et al., 530/350, 351, 399; 930/140 [IMAGE AVAILABLE]

US PAT NO: 5,571,893 [IMAGE AVAILABLE] L16: 83 of 100
REL-US-DATA: Continuation of Ser. No. 233,609, Apr. 25, 1994, Pat. No. 5,534,615.

ABSTRACT:
Isolated CHF, isolated DNA encoding CHF, and recombinant or synthetic methods of preparing CHF are disclosed, These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

84. 5,571,688, Nov. 5, 1996, Method of detecting gene expression; John J. Mekalanos, et al., 435/29, 6, 34 [IMAGE AVAILABLE]

US PAT NO: 5,571,688 [IMAGE AVAILABLE] L16: 84 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 127,905, Sep. 28, 1993, which is a continuation-in-part of Ser. No. 58,299, May 6, 1993, Pat. No. 5,434,065.

ABSTRACT:
A reporter system relating to in vivo expression technology was devised to aid in the identification and study of genes that display temporal or spatial patterns of expression during infection of host tissues. The method of this invention comprises constructing a strain or pool of strains of a microorganism which contains an artificial cointegrate comprising a reporter gene flanked by direct repeats of sequences to which a resolvase enzyme binds, thus catalyzing excision of the reporter gene, and further contains a coding sequence under the control of a promoter sequence which encodes transcripts, the expression of which are easily monitored in vitro and which result in a permanent genetic change, excision of the reporter gene, that is heritable and easily detectable subsequent to induction of the synthetic operon.

85. 5,571,675, Nov. 5, 1996, Detection and amplification of candiotrophin-1(cardiac hypertrophy factor); Joffre Baker, et al., 435/6, 91.2, 91.21; 536/24.3, 24.31, 24.32, 24.33 [IMAGE AVAILABLE]

US PAT NO: 5,571,675 [IMAGE AVAILABLE] L16: 85 of 100
REL-US-DATA: Division of Ser. No. 286,304, Aug. 5, 1994, which is a continuation-in-part of Ser. No. 233,609, Apr. 25, 1994.

ABSTRACT:
Isolated CHF, isolated DNA encoding CHF, and recombinant or synthetic methods of preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly,

these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

86. 5,543,319, Aug. 6, 1996, Recombination-proficient avian/mammalian microcell hybrids; R. E. Keith Fournier, et al., 435/349; 424/93.3; 435/70.2 [IMAGE AVAILABLE]

US PAT NO: 5,543,319 [IMAGE AVAILABLE] L16: 86 of 100

ABSTRACT:
An avian/mammalian microcell hybrid immortalized pre B cell line containing a mammalian chromosome that carries a selectable marker has been produced. The avian/mammalian microcell hybrid immortalized pre B cell line can be used for introducing predetermined point mutations into specific mammalian chromosomal loci via high frequency homologous recombination.

87. 5,541,098, Jul. 30, 1996, Urate oxidase activity protein, recombinant gene coding therefor, expression vector; micro-organisms and transformed cells; Daniel Caput, et al., 435/191, 252.33, 254.21, 320.1, 365; 536/23.2 [IMAGE AVAILABLE]

US PAT NO: 5,541,098 [IMAGE AVAILABLE] L16: 87 of 100
REL-US-DATA: Division of Ser. No. 920,519, Jul. 28, 1992, Pat. No. 5,382,518, which is a continuation of Ser. No. 659,408, Apr. 25, 1991, abandoned.

ABSTRACT:
The invention concerns a new urate oxidase activity protein which has the following sequence (SEQ ID NO:1): ##STR1## optionally preceded by a methionine, or in that it may present a degree of substantial homology with this sequence.
The invention is also aimed at medicines containing this protein as well as the genetic engineering implements to obtain it.

88. 5,534,615, Jul. 9, 1996, Cardiac hypertrophy factor and uses therefor; Joffre Baker, et al., 530/350; 424/569, 570; 530/380 [IMAGE AVAILABLE]

US PAT NO: 5,534,615 [IMAGE AVAILABLE] L16: 88 of 100

ABSTRACT:
Isolated CHF, isolated DNA encoding CHF, and recombinant or synthetic methods of preparing CHF are disclosed. These CHF molecules are shown to influence hypertrophic activity and neurological activity. Accordingly, these compounds or their antagonists may be used for treatment of heart failure, arrhythmic disorders, inotropic disorders, and neurological disorders.

89. 5,527,695, Jun. 18, 1996, Controlled modification of eukaryotic genomes; Thomas K. Hodges, et al., 800/291; 435/320.1 [IMAGE AVAILABLE]

US PAT NO: 5,527,695 [IMAGE AVAILABLE] L16: 89 of 100

ABSTRACT:
DNA constructs are provided for the creation of transgenic eukaryotic cells. These DNA constructs allow a more precise and effective transformation procedure by enabling the targeting of DNA sequences for insertion into a particular DNA locus, while enabling the removal of any randomly inserted DNA sequences that occur as a by product of known transformation procedures.

90. 5,501,967, Mar. 26, 1996, Process for the site-directed integration of DNA into the genome of plants; Remko Offringa, et al., 435/469, 252.2, 252.3, 320.1; 536/23.2, 23.6 [IMAGE AVAILABLE]

US PAT NO: 5,501,967 [IMAGE AVAILABLE] L16: 90 of 100
REL-US-DATA: Continuation of Ser. No. 659,288, May 21, 1991, abandoned.

ABSTRACT:
The present invention provides a method for site-directed integration of DNA-sequences into the genome of plants via homologous recombination, by transforming said plants using the DNA-transfer system of Agrobacterium, in which the transforming DNA comprises in its most simple form a region homologous to the target locus, as well as a region which is different from the target locus either next to one or between two T-DNA borders. Special constructs are provided, which in its most complete form have the following general structure, ##STR1## in which box 1 and 7 represent T-DNA borders, boxes 2 and 6 comprise functional expression cassettes containing negative selection genes, box 3 provides a region of homology with the target locus promoting recombination, box 4 represents a DNA sequence containing a mutation with respect to the target locus, box 5 represents a functional expression cassette containing a positive selection gene, and box E comprises a DNA sequence which is homologous to a region adjacent of the target locus, or in the vicinity of the target locus, which promotes homologous recombination.

91. 5,482,856, Jan. 9, 1996, Production of chimeric antibodies by homologous recombination; H. Perry Fell, Jr., et al., 435/320.1, 69.1, 69.6; 530/387.3, 388.8 [IMAGE AVAILABLE]

US PAT NO: 5,482,856 [IMAGE AVAILABLE] L16: 91 of 100
REL-US-DATA: Division of Ser. No. 468,035, Jan. 22, 1990, Pat. No. 5,202,238, which is a continuation-in-part of Ser. No. 243,873, Sep. 14, 1988, Pat. No. 5,204,244, which is a continuation-in-part of Ser. No. 113,800, Oct. 27, 1987, abandoned.

ABSTRACT:
A process for producing chimeric antibodies using novel recombinant DNA vectors and homologous recombination in vivo is described. The recombinant DNA constructs of the invention can be used to transfect antibody producing cells so that targeted homologous recombination occurs in the transfected cells leading to gene modification and the production of chimeric antibody molecules by the transfected cells.

92. 5,477,002, Dec. 19, 1995, Anther-specific CDNA sequences, genomic DNA sequences and recumbinant DNA sequences; Annmarie B. Tuttle, et al., 800/303; 435/320.1; 536/23.1, 23.5, 23.6, 23.7, 24.1; 800/317.3 [IMAGE AVAILABLE]

US PAT NO: 5,477,002 [IMAGE AVAILABLE] L16: 92 of 100
REL-US-DATA: Continuation of Ser. No. 908,242, Jul. 2, 1992, abandoned.

ABSTRACT:
cDNA sequences are disclosed which are expressed specifically in the anther of a plant. Genomic DNA sequences corresponding to the cDNA clones are obtained using the cDNA clones as hybridization probes. Recombinant, or chimeric, DNA sequences are constructed in which the promoter sequence from anther-specific genomic clones are operatively linked to a DNA sequence coding for a desired polypeptide. Transgenic plants are made in which the chimeric DNA sequences are expressed in the anther of the transgenic plant. In a preferred embodiment, the coding DNA sequence expresses a polypeptide which will disrupt formation of viable pollen, resulting in a male-sterile plant.

93. 5,441,884, Aug. 15, 1995, Bacillus thuringiensis transposon TN5401; James A. Baum, 435/252.31; 424/93.2; 435/252.3, 252.33, 320.1; 536/23.1, 23.2, 23.7, 24.1 [IMAGE AVAILABLE]

US PAT NO: 5,441,884 [IMAGE AVAILABLE] L16: 93 of 100

ABSTRACT:
A transposable element, or transposon, isolated from Bacillus thuringiensis (B.t.) and designated as transposon Tn5401. The invention also includes a method of using this transposon in a site-specific recombination system for construction of recombinant B.t. strains that contain insecticidal B.t. toxin protein genes and that are free of DNA not native to B.t.

94. 5,382,518, Jan. 17, 1995, Urate oxidase activity protein, recombinant gene coding therefor, expression vector, micro-organisms and transformed cells; Daniel Caput, et al., 435/191, 69.1 [IMAGE AVAILABLE]

US PAT NO: 5,382,518 [IMAGE AVAILABLE] L16: 94 of 100
REL-US-DATA: Continuation of Ser. No. 659,408, Apr. 25, 1991, abandoned.

ABSTRACT:

The invention concerns a new urate oxidase activity protein which has the following sequence:

Ser Ala Val Lys Ala Ala Arg Tyr Gly

Lys Asp Asn Val Arg Val Tyr Lys Val His
Lys Asp Glu Lys Thr Gly Val Gln Thr Val
Tyr Glu Met Thr Val Cys Val Leu Leu Glu
Gly Glu Ile Glu Thr Ser Tyr Thr Lys Ala
Asp Asn Ser Val Ile Val Ala Thr Asp Ser
Ile Lys Asn Thr Ile Tyr Ile Thr Ala Lys
Gln Asn Pro Val Thr Pro Pro Glu Leu Phe
Gly Ser Ile Leu Gly Thr His Phe Ile Glu
Lys Tyr Asn His Ile His Ala Ala His Val
Asn Ile Val Cys His Arg Trp Thr Arg Met
Asp Ile Asp Gly Lys Pro His Pro His Ser
Phe Ile Arg Asp Ser Glu Glu Lys Arg Asn
Val Gln Val Asp Val Val Glu Gly Lys Gly
Ile Asp Ile Lys Ser Ser Leu Ser Gly Leu
Thr Val Leu Lys Ser Thr Asn Ser Gln Phe
Trp Gly Phe Leu Arg Asp Glu Tyr Thr Thr
Leu Lys Glu Thr Trp Asp Arg Ile Leu Ser
Thr Asp Val Asp Ala Thr Trp Gln Trp Lys
Asn Phe Ser Gly Leu Gln Glu Val Arg Ser
His Val Pro Lys Phe Asp Ala Thr Trp Ala
Thr Ala Arg Glu Val Thr Leu Lys Thr Phe
Ala Glu Asp Asn Ser Ala Ser Val Gln Ala
Thr Met Tyr Lys Met Ala Glu Gln Ile Leu
Ala Arg Gln Gln Leu Ile Glu Thr Val Glu
Tyr Ser Leu Pro Asn Lys His Tyr Phe Glu
Ile Asp Leu Ser Trp His Lys Gly Leu Gln
Asn Thr Gly Lys Asn Ala Glu Val Phe Ala
Pro Gln Ser Asp Pro Asn Gly Leu Ile Lys
Cys Thr Val Gly Arg Ser Ser Leu Lys Ser
Lys Leu

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optionally preceeded by a methionine, or in that it may present a degree of substantial homology with this sequence.
The invention is also aimed at medicines containing this protein as well as the genetic engineering implements to obtain it.

95. 5,334,515, Aug. 2, 1994, Method for altering a nucleotide sequence; Ayoub Rashtchian, et al., 435/91.2, 91.41, 91.51, 227 [IMAGE AVAILABLE]

US PAT NO: 5,334,515 [IMAGE AVAILABLE] L16: 95 of 100
REL-US-DATA: Continuation of Ser. No. 683,684, Apr. 10, 1991, abandoned.

ABSTRACT:
A method and kit, employing exo-sample nucleotides such as deoxyuridine, capable of altering the nucleic acid sequence present at the 3' or 5' end of a DNA or RNA molecule is provided. The method and kit can be used to achieve the selective amplification of nucleic acid molecules.

96. 5,272,071, Dec. 21, 1993, Method for the modification of the expression characteristics of an endogenous gene of a given cell line; Scott C. Chappel, 435/6, 69.1, 252.3, 320.1, 463, 464; 536/23.1, 24.3 [IMAGE AVAILABLE]

US PAT NO: 5,272,071 [IMAGE AVAILABLE] L16: 96 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 454,783, Dec. 22, 1989, abandoned.

ABSTRACT:

Normally transcriptionally silent genes in a cell line or microorganism may be activated for expression by inserting a DNA regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell or which is promiscuous, the regulatory element being inserted so as to be operatively linked with the normally silent gene in question. The insertion is accomplished by means of homologous recombination by creating a DNA construct including a segment having a DNA segment of the normally silent gene (targeting DNA) and the DNA regulatory element to induce gene transcription. The technique is also used to modify the expression characteristics of any endogenous gene of a given cell line or microorganism.

97. 5,268,285, Dec. 7, 1993, Strains of yeast with increased rates of glycolysis; David T. Rogers, et al., 435/161, 194, 254.21, 320.1, 483 [IMAGE AVAILABLE]

US PAT NO: 5,268,285 [IMAGE AVAILABLE] L16: 97 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 533,992, Jun. 4, 1990, abandoned, which is a continuation of Ser. No. 85,099, Jul. 7, 1987, abandoned, which is a continuation-in-part of Ser. No. 796,551, Nov. 8, 1985, abandoned.

ABSTRACT:
The present invention provides a process for increasing the rate of production of carbon dioxide, ethanol and other fermentation products such as citric acid, produced by yeast such as Saccharomyces cerevisiae during fermentation, and decreasing biomass production by regulating the rate of glycolysis indirectly through changing the energy balance of the cell, i.e., by reducing intracellular ATP levels. Modifications for so altering the glycolysis rate involve the use of either a regulated ATP hydrolysis within the cell or a regulated leakage of ATP from the cell. This invention encompasses several ways for altering the yeast ATP level including (a) engaging futile metabolic cycles to increase ATP consumption; and (b) using an altered exocellular acid phosphatase so that it becomes intracellular to increase intracellular ATP hydrolysis; by which alterations in the ATP level may be turned off during growth of the yeast on a commercial scale, and then turned on before or during, and preferably before or at a very early stage of, the dough-rising phase.

98. 5,202,238, Apr. 13, 1993, Production of chimeric antibodies by homologous recombination; H. Perry Fell, Jr., et al., 435/69.6, 69.7, 70.1, 70.21, 320.1, 463, 465; 530/387.3, 388.8; 536/23.53 [IMAGE AVAILABLE]

US PAT NO: 5,202,238 [IMAGE AVAILABLE] L16: 98 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 243,873, Sep. 14, 1988, and a continuation-in-part of Ser. No. 113,800, Oct. 27, 1987, abandoned.

ABSTRACT:
A process for producing chimeric antibodies using novel recombinant DNA vectors and homologous recombination in vivo is described. The recombinant DNA constructs of the invention can be used to transfect antibody producing cells so that targeted homologous recombination occurs in the transfected cells leading to gene modification and the production of chimeric antibody molecules by the transfected cells.

99. 5,185,250, Feb. 9, 1993, Human .gamma., .delta.T cell antigen receptor polypeptides and nucleic acids; Michael B. Brenner, et al., 435/69.3, 7.24, 69.1; 530/350, 387.9, 388.22, 388.75; 536/23.5 [IMAGE AVAILABLE]

US PAT NO: 5,185,250 [IMAGE AVAILABLE] L16: 99 of 100
REL-US-DATA: Continuation-in-part of Ser. No. 187,698, Apr. 29, 1988, which is a continuation-in-part of Ser. No. 115,256, Oct. 29, 1987, Pat. No. 5,024,940, which is a continuation-in-part of Ser. No. 16,252, Feb. 19, 1987, which is a continuation-in-part of Ser. No. 882,100, Jul. 3, 1986, abandoned.

ABSTRACT:
The present invention is directed to purified polypeptides comprising the .gamma. T cell antigen receptor (TCR) polypeptide, the .delta. TCR polypeptide, a .gamma., .delta. TCR complex, or a fragment thereof containing an epitope. The invention also relates to nucleic acid sequences encoding such polypeptides, and subsequences thereof. In specific embodiments, the invention relates to nucleic acid sequences comprising variable, diversity, joining, or constant regions of the .delta. TCR gene sequence. The invention also relates to monoclonal antibodies specifically reactive with an epitope of the gamma or delta TCR polypeptides. In specific embodiments, these antibodies are reactive with the delta constant region, the delta variable region, or gamma constant region. Such antibodies can be identified by detecting co-modulation of the CD3 antigen. In another embodiment, the invention relates to compositions comprising substantially purified cells which express both a .gamma., .delta. TCR and the CD4 antigen. The invention also relates to a composition comprising cells which express a .gamma., .delta. TCR that is not associated with a CD3 complex.

100. 5,159,066, Oct. 27, 1992, Recombination activating gene (RAG-1); David G. Schatz, et al., 536/23.2, 23.5 [IMAGE AVAILABLE]

US PAT NO: 5,159,066 [IMAGE AVAILABLE] L16: 100 of 100

ABSTRACT:
Recombination activating gene of mammalian origin (RAG-1), cDNA of RAG-1
of mammalian origin, mRNA expressed by RAG-1, the encoded recombinase

and
antibodies specific for the recombinase, as well as the use of the same
for a diagnostic or therapeutic purpose.

=> log y

U.S. Patent & Trademark Office LOGOFF AT 12:35:57 ON 17 MAY 1999

09/203502
AA#5

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   Set  Items  Description
   ---  -----  -----------
? s dhfr

    S1   5329  DHFR
? s recombinase??

    S2   4603  RECOMBINASE??
? s s1 and s2

        5329  S1
        4603  S2
    S3      0  S1 AND S2
? s homologous(w)recombination

      213859  HOMOLOGOUS
      133841  RECOMBINATION
    S4  11756  HOMOLOGOUS(W)RECOMBINATION
? s s1 and s4

        5329  S1
       11756  S4
    S5     38  S1 AND S4
? s s2 and s4

        4603  S2
       11756  S4
    S6    248  S2 AND S4
? s marker

    S7 278714  MARKER
? s s4(10n)s7

       11756  S4
      278714  S7
    S8    292  S4(10N)S7
? s s2 and s8

        4603  S2
         292  S8
    S9     20  S2 AND S8
? s s5 or s9

          38  S5
          20  S9
   S10    58  S5 OR S9
? rd

...examined 50 records  (50)
...completed examining records
   S11    32  RD (unique items)
? s s10 and py>=1997

          58  S10
     4276252  PY>=1997
   S12    17  S10 AND PY>=1997
? t s12/3,ab/1-17


 12/3,AB/1    (Item 1 from file: 5)
DIALOG(R)File  5:Biosis Previews(R)
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11489906  BIOSIS NO.: 199800271238
DNA cassette exchange in ES cells mediated by FLP %%%recombinase%%%: An
 efficient strategy for repeated modification of tagged loci by
 marker-free constructs.

AUTHOR: Seibler Jost; Schuebeler Dirk; Fiering Steven; Groudine Mark; Bode
 Juergen(a)
AUTHOR ADDRESS: (a)GBF, Natl. Res. Cent. Biotechnol., Mascheroder Weg 1,
 D-38124 Braunschweig, Germany

JOURNAL: Biochemistry  37 (18):p6229-6234 May 5, 1998
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The repeated modification of a genomic locus is a technically
 demanding but powerful strategy to analyze the function of a particular
 gene product or the role of cis-regulatory DNA elements in mammalian
 cells. The initial step is "tagging" a site with a selectable
 %%%marker%%% which is done by %%%homologous%%%
%%%recombination%%% (HR)
 to modify a known locus or by random integration to study cis-regulatory
 elements at a reproducibly accessible genomic location. The tag is then
 used to target the construct of choice during an exchange step.
 Presented here is a novel technique in which the exchange is independent
 of HR and does not introduce vector sequences. It relies on our previous
 studies on the replacement of DNA cassettes by
FLP-%%%recombinase%%%,
 whereby some common limitations can be overcome. To this end, the tag, a
 hygtk positive/negative selection marker, is integrated into the genome
 of embryonic stem (ES) cells. This marker is flanked by a wild-type
 Flp-recognition target (FRT) site on one end and by a modified
 heterospecific FRT site on the other. Successful Flp-mediated replacement
 of the hygtk cassette is enriched by ganciclovir (GANC) selection for
 cells that lack the encoded fusion protein. Thereby, the hygtk gene can
 be exchanged for virtually any sequence in a single efficient step
 without the need of introducing a positive selectable marker. The system
 can hence be used to analyze the function of either a gene product or
 regulatory sequences in ES cells or the transgenic mice derived thereof.


 12/3,AB/2    (Item 2 from file: 5)
DIALOG(R)File  5:Biosis Previews(R)
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11295630  BIOSIS NO.: 199800076962
Transfection of malaria parasites.

AUTHOR: Waters A P(a); Thomas A W; Van Dijk M R(a); Janse C J(a)
AUTHOR ADDRESS: (a)Dep. Parasitol., Univ. Leiden, Postbox 9605, 2300 RC
 Leiden, Netherlands

JOURNAL: Methods (Orlando)  13 (2):p134-147 Oct., 1997
ISSN: 1046-2023
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The stable genetic transformation of three phylogenetically
 diverse species of Plasmodium, the parasitic etiological agent of
 malaria, is now possible. The parasite is haploid throughout the vast
 majority of its life cycle. Therefore with the single selectable marker
 activity and protocols currently available, it is possible not only to
 express introduced transgenes but also to study the effects of
 site-specific %%%homologous%%% %%%recombination%%% such as gene
knockout.
 Transgene expression will allow the detailed study of many aspects of the
 cellular biology of malaria parasites, for example, the mechanisms
 underlying drug resistance and protein trafficking. We describe here the
 methods for propagation of the two animal models (Plasmodium berghei and
 Plasmodium knowlesi) and for transfection of these two species and the
 human parasite, Plasmodium falciparum. Examples of transgene expression
 are given.


 12/3,AB/3    (Item 3 from file: 5)
DIALOG(R)File  5:Biosis Previews(R)
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11295628  BIOSIS NO.: 199800076960
Tagging genes and trapping promoters in Toxoplasma gondii by insertional
 mutagenesis.

AUTHOR: Roos David S(a); Sullivan William J; Striepen Boris; Bohne Wolfgang
 ; Donald Robert G K
AUTHOR ADDRESS: (a)Dep. Biol., Univ., Pennsylvania, 415 S. University Ave.,
 Philadelphia, PA 19104-6018, USA
```

JOURNAL: Methods (Orlando) 13 (2):p112-122 Oct., 1997
ISSN: 1046-2023
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Plasmid vectors that incorporate sequence elements from the dehydrofolate reductase-thymidylate synthase (%%%DHFR%%%-TS) locus of Toxoplasma gondii integrate into the parasite genome with remarkably high frequency (>1% of transfected parasites). These vectors may-but need not-include mutant %%%DHFR%%%-TS alleles that confer pyrimethamine resistance to transgenic parasites. Large genomic constructs integrate at the endogenous locus by %%%homologous%%% %%%recombination%%%, but cDNA-derived sequences lacking long stretches of contiguous genomic DNA (due to intron excision) typically integrate into chromosomal DNA by nonhomologous recombination. Nonhomologous integration occurs effectively at random; and coupled with the high frequency of transformation, this allows a large fraction of the parasite genome to be tagged in a single electroporation cuvette. Genomic tagging permits insertional mutagenesis studies conceptually analogous to transposon mutagenesis in bacteria, yeast, Drosophila, etc. In theory (and, thus far, in practice), this allows identification of any gene whose inactivation is not lethal to the haploid tachyzoite form of T. gondii and for which a suitable selection or screen is available. Transformation vectors can be engineered to facilitate rescue of the tagged locus and to include a variety of reporters or selectable markers. Genetic strategies are also possible, using reporters whose function can be assayed by metabolic, visual, or immunological screens to "trap" genes that are activated (or inactivated) under various conditions of interest.

12/3,AB/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10932707 BIOSIS NO.: 199799553852
Transfection of the primate malaria parasite Plasmodium knowlesi using entirely heterologous constructs.

AUTHOR: Van Der Wel Anna M Tomas; Kocken Clemens H M; Malhotra Pawan; Janse Chris J; Waters Andrew P; Thomas Alan W(a)
AUTHOR ADDRESS: (a)Dep. Parastiol., Biomed. Primate Research, Centre, Lange Kleiweg 151, 2288 GJ Rijwijk, Netherlands

JOURNAL: Journal of Experimental Medicine 185 (8):p1499-1503 1997
ISSN: 0022-1007
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The recently developed transfection systems for Plasmodium berghei and Plasmodium falciparum offer important new tools enabling further insight into the biology of malaria parasites. These systems rely upon artificial parasite-host combinations which do not allow investigation into the complex interactions between parasites and their natural hosts. Here we report on stable transfection of Plasmodium knowlesi (a primate malaria parasite that clusters phylogenetically with P. vivax) for which both natural and artificial experimental hosts are available. Transfection of this parasite offers the opportunity to further analyze the biology of antigens not only in a natural host but also in hosts that are closely related to humans. To facilitate future development of integration-dependent transfection in P. knowlesi, completely heterologous plasmids that would reduce %%%homologous%%% %%%recombination%%% at unwanted sites in the genome were constructed. These plasmids contained the pyrimethamine-resistant form of dihydrofolate reductase-thymidylate synthase (%%%dhfr%%%-ts) from Toxoplasma gondii or P. berghei, under control of either (a) P. berghei or (b) P. falciparum promoters. Plasmids were electroporated into mature P. knowlesi schizonts and these cells were injected into rhesus monkeys (Macaca mulatta). After pyrimethamine treatment of these monkeys, resistant parasites were obtained that contained the plasmids. Promoter regions of both P. berghei and P. falciparum controlling %%%dhfr%%%-ts expression were effective in conferring pyrimethamine resistance in P. knowlesi, indicating that common signals control gene expression in phylogenetically distant Plasmodium species.

12/3,AB/5 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
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07267544 EMBASE No: 1998156315
DNA cassette exchange in ES cells mediated by FLP %%%recombinase%%%: An efficient strategy for repeated modification of tagged loci by marker-free constructs
Seibler J.; Schubeler D.; Fiering S.; Groudine M.; Bode J.
J. Bode, GBF, Natl. Res. Center for Biotechnology, Dartmouth Medical School, Mascheroder Weg 1, D-38124 Braunschweig Germany
Biochemistry (BIOCHEMISTRY) (United States) 05 MAY 1998, 37/18 (6229-6234)

CODEN: BICHA ISSN: 0006-2960
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 25

The repeated modification of a genomic locus is a technically demanding but powerful strategy to analyze the function of a particular gene product or the role of cis-regulatory DNA elements in mammalian cells. The initial step is 'tagging' a site with a selectable %%%marker%%% which is done by %%%homologous%%% %%%recombination%%% (HR) to modify a known locus or by random integration to study cis-regulatory elements at a reproducibly accessible genomic location. The tag is then used to target the construct of choice during an exchange step. Presented here is a novel technique in which the exchange is independent of HR and does not introduce vector sequences. It relies on our previous studies on the replacement of DNA cassettes by FLP-%%%recombinase%%%, whereby some common limitations can be overcome. To this end, the tag, a hygtk positive/negative selection marker, is integrated into the genome of embryonic stem (ES) cells. This marker is flanked by a wild-type Flp-recognition target (FRT) site on one end and by a modified heterospecific FRT site on the other. Successful Flp-mediated replacement of the hygtk cassette is enriched by ganciclovir (GANC) selection for cells that lack the encoded fusion protein. Thereby, the hygtk gene can be exchanged for virtually any sequence in a single efficient step without the need of introducing a positive selectable marker. The system can hence be used to analyze the function of either a gene product or regulatory sequences in ES cells or the transgenic mice derived thereof.

12/3,AB/6 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE
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07133509 EMBASE No: 1998022357
Tagging genes and trapping promoters in Toxoplasma gondii by insertional mutagenesis
Roos D.S.; Sullivan W.J.; Striepen B.; Bohne W.; Donald R.G.E.
D.S. Roos, Department of Biology, University of Pennsylvania, 415 South University Avenue, Philadelphia, PA 19104-6018 United States
AUTHOR EMAIL: droos@sas.upenn.edu
Methods: A Companion to Methods in Enzymology (METHODS COMPANION METHODS ENZYMOL.) (United States) 1997, 13/2 (112-122)

CODEN: MTHDE ISSN: 1046-2023
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 30

Plasmid vectors that incorporate sequence elements from the dehydrofolate reductase-thymidylate synthase (%%%DHFR%%%-TS) locus of Toxoplasma gondii integrate into the parasite genome with remarkably high frequency (> 1% of transfected parasites). These vectors may - but need not - include mutant %%%DHFR%%%-TS alleles that confer pyrimethamine resistance to transgenic parsites. Large genomic constructs integrate at the endogenous locus by %%%homologous%%% %%%recombination%%%, but cDNA-derived sequences lacking long stretches of contiguous genomic DNA (due to intron excision) typically integrate into chromosomal DNA by nonhomologous recombination. Nonhomologous integration occurs effectively at random; and coupled with

the high frequency of transformation, this allows a large fraction of the parasite genome to be tagged in a single electroporation cuvette. Genomic tagging permits insertional mutagenesis studies conceptually analogous to transposon mutagenesis in bacteria, yeast, Drosophila, etc. In theory (and, thus far, in practice), this allows identification of any gene whose inactivation is not lethal to the haploid tachyzoite form of T. gondii and for which a suitable selection or screen is available. Transformation vectors can be engineered to facilitate rescue of the tagged locus and to include a variety of reporters or selectable markers. Genetic strategies are also possible, using reporters whose function can be assayed by metabolic, visual, or immunological screens to 'trap' genes that are activated (or inactivated) under various conditions of interest.

12/3,AB/7 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE
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06849190 EMBASE No: 1997131778
Transfection of the primate malaria parasite Plasmodium knowlesi using entirely heterologous constructs
Van der Wel A.M.; Tomas A.M.; Kocken C.H.M.; Malhotra P.; Janse C.J.; Waters A.P.; Thomas A.W.
A.W. Thomas, Department of Parasitology, Biomedical Primate Research Centre, Lange Kleiweg 151, 2288 GJ Rijswijk Netherlands
Journal of Experimental Medicine (J. EXP. MED.) (United States) 1997, 185/8 (1499-1503)

CODEN: JEMEA ISSN: 0022-1007
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 23

The recently developed transfection systems for Plasmodium berghei and Plasmodium falciparum offer important new tools enabling further insight into the biology of malaria parasites. These systems rely upon artificial parasite-host combinations which do not allow investigation into the complex interactions between parasites and their natural hosts. Here we report on stable transfection of Plasmodium knowlesi (a primate malaria parasite that cluster phylogenetically with P. vivax) for which both natural and artificial experimental hosts are available. Transfection of this parasite offers the opportunity to further analyze the biology of antigens not only in a natural host but also in hosts that are closely related to humans. To facilitate future development of integration-dependent transfection in P. knowlesi, completely heterologous plasmids that would reduce %%%homologous%%% %%%recombination%%% at unwanted
sites in the genome were constructed. These plasmids contained the pyrimethamine-resistant form of dihydrofolate reductase-thymidylate synthase (%%%dhfr%%%-ts) from Toxoplasma gondii or P. berghei, under control of either (a) P. berghei or (b) P. falciparum promoters. Plasmids were electroporated into mature P. knowlesi schizonts and these cells were injected into rhesus monkeys (Macaca mulatta). After pyrimethamine treatment of these monkeys, resistant parasites were obtained that continued the plasmids. Promoter regions of both P. berghei and P. falciparum controlling %%%dhfr%%%-ts expression were effective in conferring pyrimethamine resistance in P. knowlesi, indicating that common signals control gene expression in phylogenetically distant Plasmodium species.

12/3,AB/8 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09517827 98241343
DNA cassette exchange in ES cells mediated by Flp %%%recombinase%%%: an
efficient strategy for repeated modification of tagged loci by marker-free constructs.
Seibler J; Schubeler D; Fiering S; Groudine M; Bode J
GBF, National Research Center for Biotechnology, Mascheroder Weg 1, D-38124 Braunschweig.
Biochemistry (UNITED STATES) May 5 %%%1998%%%, 37 (18) p6229-34,
ISSN 0006-2960 Journal Code: A0G
Contract/Grant No.: CA54337, CA, NCI; DK44746, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The repeated modification of a genomic locus is a technically demanding but powerful strategy to analyze the function of a particular gene product or the role of cis-regulatory DNA elements in mammalian cells. The initial step is "tagging" a site with a selectable %%%marker%%% which is done by %%%homologous%%% %%%recombination%%% (HR) to modify a known locus or by
random integration to study cis-regulatory elements at a reproducibly accessible genomic location. The tag is then used to target the construct of choice during an exchange step. Presented here is a novel technique in which the exchange is independent of HR and does not introduce vector sequences. It relies on our previous studies on the replacement of DNA cassettes by FLP-%%%recombinase%%%, whereby some common limitations can be
overcome. To this end, the tag, a hygtk positive/negative selection marker, is integrated into the genome of embryonic stem (ES) cells. This marker is flanked by a wild-type Flp-recognition target (FRT) site on one end and by a modified heterospecific FRT site on the other. Successful Flp-mediated replacement of the hygtk cassette is enriched by ganciclovir (GANC) selection for cells that lack the encoded fusion protein. Thereby, the hygtk gene can be exchanged for virtually any sequence in a single efficient step without the need of introducing a positive selectable marker. The system can hence be used to analyze the function of either a gene product or regulatory sequences in ES cells or the transgenic mice derived thereof.

12/3,AB/9 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09354238 98071021
Tagging genes and trapping promoters in Toxoplasma gondii by insertional mutagenesis.
Roos DS; Sullivan WJ; Striepen B; Bohne W; Donald RG
Department of Biology, University of Pennsylvania, 415 South University Avenue, Philadelphia, Pennsylvania, 19104-6018, USA. droos@sas.upenn.edu
Methods (UNITED STATES) Oct %%%1997%%%, 13 (2) p112-22,
ISSN
1046-2023 Journal Code: CPO
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Plasmid vectors that incorporate sequence elements from the dehydrofolate reductase-thymidylate synthase (%%%DHFR%%%-TS) locus of Toxoplasma gondii
integrate into the parasite genome with remarkably high frequency (>1% of transfected parasites). These vectors may-but need not-include mutant %%%DHFR%%% -TS alleles that confer pyrimethamine resistance to transgenic
parasites. Large genomic constructs integrate at the endogenous locus by %%%homologous%%% %%%recombination%%%, but cDNA-derived sequences lacking
long stretches of contiguous genomic DNA (due to intron excision) typically integrate into chromosomal DNA by nonhomologous recombination. Nonhomologous integration occurs effectively at random; and coupled with the high frequency of transformation, this allows a large fraction of the parasite genome to be tagged in a single electroporation cuvette. Genomic tagging permits insertional mutagenesis studies conceptually analogous to transposon mutagenesis in bacteria, yeast, Drosophila, etc. In theory (and, thus far, in practice), this allows identification of any gene whose inactivation is not lethal to the haploid tachyzoite form of T. gondii and for which a suitable selection or screen is available. Transformation vectors can be engineered to facilitate rescue of the tagged locus and to include a variety of reporters or selectable markers. Genetic strategies are also possible, using reporters whose function can be assayed by metabolic, visual, or immunological screens to "trap" genes that are activated (or inactivated) under various conditions of interest. Copyright 1997 Academic Press.

12/3,AB/10 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09043326 97272086
Transfection of the primate malaria parasite Plasmodium knowlesi using entirely heterologous constructs.
van der Wel AM; Tomas AM; Kocken CH; Malhotra P; Janse CJ; Waters AP;
Thomas AW

Department of Parasitology, Biomedical Primate Research Centre, Rijswijk, The Netherlands.
J Exp Med (UNITED STATES) Apr 21 %%%1997%%%, 185 (8) p1499-503, ISSN
0022-1007 Journal Code: I2V
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The recently developed transfection systems for Plasmodium berghei and Plasmodium falciparum offer important new tools enabling further insight into the biology of malaria parasites. These systems rely upon artificial parasite-host combinations which do not allow investigation into the complex interactions between parasites and their natural hosts. Here we report on stable transfection of Plasmodium knowlesi (a primate malaria parasite that clusters phylogenetically with P. vivax) for which both natural and artificial experimental hosts are available. Transfection of this parasite offers the opportunity to further analyze the biology of antigens not only in a natural host but also in hosts that are closely related to humans. To facilitate future development of integration-dependent transfection in P. knowlesi, completely heterologous plasmids that would reduce %%%homologous%%% %%%recombination%%% at unwanted
sites in the genome were constructed. These plasmids contained the pyrimethamine-resistant form of dihydrofolate reductase-thymidylate synthase (%%%dhfr%%% -ts) from Toxoplasma gondii or P. berghei, under
control of either (a) P. berghei or (b) P. falciparum promoters. Plasmids were electroporated into mature P. knowlesi schizonts and these cells were injected into rhesus monkeys (Macaca mulatta). After pyrimethamine treatment of these monkeys, resistant parasites were obtained that contained the plasmids. Promoter regions of both P. berghei and P. falciparum controlling %%%dhfr%%% -ts expression were effective in conferring pyrimethamine resistance in P. knowlesi, indicating that common signals control gene expression in phylogenetically distant Plasmodium species.

12/3,AB/11 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0221504 DBA Accession No.: 98-03101 PATENT
DNA construct for transforming yeast - containing a self-eliminating combination of a %%%recombinase%%% gene and a selectable marker
AUTHOR: Ashikari T; Kondo H; Sakakibara K; Araki H; Oshima Y
CORPORATE SOURCE: Osaka, Japan.
PATENT ASSIGNEE: Suntory %%%1997%%%
PATENT NUMBER: EP 814165 PATENT DATE: 971229 WPI ACCESSION NO.: 98-044341
(9805)
PRIORITY APPLIC. NO.: JP 96179820 APPLIC. DATE: 960621
NATIONAL APPLIC. NO.: EP 97304306 APPLIC. DATE: 970616
LANGUAGE: English
ABSTRACT: A new DNA construct (in the form of a plasmid) contains: a DNA
fragment capable of recombination with chromosomal DNA; a %%%recombinase%%%-sensitive (RS) sequence; a recombinant gene under the
control of an inducible promoter; a selectable marker; a second RS sequence; and a second DNA fragment capable of recombination with chromosomal DNA. The RS sequences are orientated in the same direction and each has a defined DNA sequence where the RS sequence nearest to the %%%recombinase%%% gene lacks 0-10 terminal nucleotides from the end
opposite to the %%%recombinase%%% gene and the RS sequence nearest to
the selectable marker lacks 0-10 terminal nucleotides from the end opposite to the selectable marker. The new DNA may be used for the transformation of yeast cells by: integrating the DNA into a yeast chromosome by recombination between the chromosomal DNA and the new DNA
fragments; selecting cells having integrated the DNA construct on the basis of expression of the selectable marker; and inducing the promoter to express the %%%recombinase%%% gene, causing recombination between
the 1st and 2nd RS sequences and elimination of the %%%recombinase%%%
gene, promoter and selectable marker. (39pp)

12/3,AB/12 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0219018 DBA Accession No.: 98-00615 PATENT
New genetic constructs for transformation of organisms, particularly plants - Cre-%%%recombinase%%% or Flp-%%%recombinase%%% co-expression with a
ribozyme, antisense RNA, sense suppression RNA or plant growth factor biosynthetic gene, in a tobacco or potato transgenic plant
AUTHOR: Surin B P; de Feyter R C; Graham M W; Waterhouse P M; Keese P K
; Shahjahan A
CORPORATE SOURCE: Campbell, Australian Capital Territory, Australia; Acton,
Australian Capital Territory, Australia.
PATENT ASSIGNEE: CSIRO; Univ.Australian-Nat. %%%1997%%%
PATENT NUMBER: WO 9737012 PATENT DATE: 971009 WPI ACCESSION NO.:
97-526087 (9748)
PRIORITY APPLIC. NO.: AU 969031 APPLIC. DATE: 960329
NATIONAL APPLIC. NO.: WO 97AU197 APPLIC. DATE: 970327
LANGUAGE: English
ABSTRACT: A new construct has a DNA cassette with a %%%recombinase%%% unit
(with a Cre-%%%recombinase%%% or Flp-%%%recombinase%%% gene, terminator
and 1st promoter) linked to a transgene unit (with 1 or more transgenes and 2nd promoters), flanked by 2 %%%recombinase%%% -binding recombination loci (e.g. lox or frt sites). The transgene may encode a ribozyme, antisense RNA, co-suppression RNA, or may be a selectable marker, reporter gene or an auxin or cytokinin biosynthetic gene or regulatory sequence (e.g. an ipt gene). An intron may be inserted to disrupt %%%recombinase%%% expression. The cassette may be expressed in
a tobacco (Nicotiana tabacum), potato (Solanum tuberosum), sweet potato, Jerusalem artichoke, taro, yam, eucalyptus, pine, aspen, gerbera, chrysanthemum, orchid, lily, rose, fuchsia, azalea, carnation, camellia, gardenia, orange, lemon, grapefruit, tangerine, lime, apple, pear, strawberry, raspberry, loganberry, blackberry, sugarcane, banana, plantain, pineapple or asparagus transgenic plant. The construct may be used for selective removal or integration of transgenes, with tight regulation of expression. (84pp)

12/3,AB/13 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0216607 DBA Accession No.: 97-11728
Cre/loxP-mediated excision of a neomycin-resistance expression unit from an integrated retroviral vector increases long terminal repeat-driven transcription in human hematopoietic cells - DNA cassette excision by homologous recombination, for improved retro virus vector-mediated gene transfer and gene therapy
AUTHOR: Fernex C; Dubreuil P; Mannoni P; +Bagnis C
CORPORATE AFFILIATE: Inst.Paoli-Calmettes INSERM
CORPORATE SOURCE: Centre de Therapie Genique, Institut Paoli-Calmettes, 232
Boulevard de Sainte-Marguerite, BP 156, 13273 Marseilles Cedex 09, France. email:bagnisc@marseille.fnclcc.fr
JOURNAL: J.Virol. (71, 10, 7533-40) %%%1997%%%
ISSN: 0022-538X CODEN: JOVIAM
LANGUAGE: English
ABSTRACT: A Cre-loxP recombination system was developed for specific
excision of a selectable marker expression unit from integrated retro viruses after gene transfer to hematopoietic cells, in order to increase target gene expression levels. A retro virus vector containing both a neomycin-resistance expression unit, flanked by loxP sites, and a granulocyte-macrophage colony stimulating factor (GM-CSF) cDNA was used to transduce a human hematopoietic K562 cell culture. Four transduced clones were then superinfected with a retro virus vector containing a Cre-%%%recombinase%%% expression unit. In 30 doubly transduced subclones, there was a strict correlation between cre expression and loxP-flanked DNA cassette excision, implying that Cre-%%%recombinase%%% activity was very efficient with retro virus vectors. Excision of the selectable marker cassette resulted in a significant increase in GM-CSF gene transcription, driven by the retro virus

promoter. This system should be useful in gene therapy, and should reduce expression of undesirable additional foreign gene products. (37 ref)

12/3,AB/14 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0211797 DBA Accession No.: 97-06918
Transfection of the primate malaria parasite Plasmodium knowlesi using entirely heterologous constructs - for use in malaria recombinant vaccine development
AUTHOR: van der Wel A M; Tomas A M; Kocken C H M; Malhotra P; Janse C J
; Waters A P; +Thomas A W
CORPORATE AFFILIATE: Biomed.Primate-Res.Cent.Rijswijk Univ.Leiden Cent.Malaria-Other-Trop.Dis.Lisbon
CORPORATE SOURCE: Department of Parasitology, Biomedical Primate Research
Centre, Lange Kleiweg 151, 2288 GJ Rijswijk, The Netherlands.
JOURNAL: J.Exp.Med. (185, 8, 1499-1503) %%%1997%%%
ISSN: 0022-1007 CODEN: JEMEAV
LANGUAGE: English
ABSTRACT: A system was developed for stable transfection of Plasmodium
knowlesi (a primate malaria parasite in the Plasmodium vivax cluster). Completely heterologous vectors (plasmid pDT.Tg23 and plasmid pchD5.1/C3) designed to reduce %%%homologous%%% %%%recombination%%% at
unwanted sites in the genome, were constructed. The plasmids contained the pyrimethamine resistant form of the dihydrofolate-reductase (EC-1.5.1.3)-thymidylate-synthase (EC-2.1.1.45) (%%%dhfr%%%-ts) gene
from Toxoplasma gondii or Plasmodium berghei, under the control of a P. berghei or Plasmodium falciparum promoter. Plasmids were introduced into mature P. knowlesi schizonts by electroporation, and recombinant cells were injected into rhesus monkeys. After pyrimethamine treatment of monkeys, resistant parasites containing plasmids were obtained. Both the P. berghei and P. falciparum promoter regions controlling %%%dhfr%%% -ts expression conferred pyrimethamine resistance in P. knowlesi, indicating that common signals controlled gene expression in phylogenetically distant Plasmodium spp. This system should be useful in malaria recombinant vaccine production. (23 ref)

12/3,AB/15 (Item 5 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0210191 DBA Accession No.: 97-05312
Ligand-inducible gene targeting in mice - tamoxifen administration to chimeric Cre-%%%recombinase%%%-expressing transgenic mouse for tkneo
selectable %%%marker%%% gene excision; %%%homologous%%% %%%recombination%%% (conference abstract)
AUTHOR: Feil R; Brocard J; Mascrez B; LeMeur M; Metzger D; Chambon P
CORPORATE AFFILIATE: CNRS INSERM Univ.Strasbourg-Louis-Pasteur
CORPORATE SOURCE: Institut de Genetique et de Biologie Moleculaire et Cellulaire, CNRS, INSERM, Universite Louis Pasteur, College de France, 67404 Illkirch-Cedex, C.U. de Strasbourg, France.
JOURNAL: Arch.Pharmacol. (355, 4, Suppl., 41) %%%1997%%%
ISSN: 0028-1298 CODEN: NSAPCC
CONFERENCE PROCEEDINGS: Deutsche Gesellschaft fuer Experimentelle und
Klinische Pharmakologie und Toxikologie, 38th Spring Meeting, Mainz, Germany, 11-13 March, 1997.
LANGUAGE: English
ABSTRACT: Current gene targeting technology does not allow gene inactivation at a given time and/or in a given tissue. To achieve conditional gene inactivation in mice, a ligand-inducible site-specific recombination system based on an engineered
Cre-%%%recombinase%%% was
set up. Cre was fused to a mutated ligand-binding domain of a human estrogen receptor supposed to bind the synthetic ligand tamoxifen but not endogenous estradiol. Transgenic mice were generated, expressing the chimeric %%%recombinase%%% (Cre-ERT) under the control of a cytomegalo virus promoter and were analyzed for the excision of a chromosomally-integrated tkneo gene flanked by loxP sites. Marker gene excision was induced by tamoxifen administration to the transgenic mice. This conditional site-specific recombination system should allow the analysis of knockout phenotypes that cannot be addressed by conventional gene targeting. (0 ref)

12/3,AB/16 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 1999 American Chemical Society. All rts. reserv.

128240335 CA: 128(20)240335t PATENT
Mammalian and human gene REC2 recombinases to induce transformation by homologous recombination and their promoters for sensitization of cells to irradiation
INVENTOR(AUTHOR): Holloman, William K.; Rice, Michael C.; Smith, Sheryl
T.; Shu, Zhigang; Kmiec, Eric B.
LOCATION: USA
ASSIGNEE: Thomas Jefferson University; Cornell Research Foundation, Inc.
PATENT: PCT International ; WO 9811214 A1 DATE: 19980319
APPLICATION: WO 97IB1217 (19970911) *US 25929 (19960911)
PAGES: 88 pp. CODEN: PIXXD2 LANGUAGE: English CLASS:
C12N-015/12A;
C12N-015/87B; C12P-019/34B DESIGNATED COUNTRIES: AL; AM; AU; AZ; BA; BB;
BG; BR; BY; CA; CN; CU; CZ; EE; GE; GH; HU; IL; IS; JP; KG; KP; KR; KZ; LC;
LK; LR; LT; LV; MD; MG; MK; MN; MX; NO; NZ; PL; RO; RU; SG; SI; SK; SL; TJ;
TM; TR; TT; UA; UZ; VN; YU; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM
DESIGNATED REGIONAL: GH; KE; LS; MW; SD; SZ; UG; ZW; AT; BE; CH; DE; DK;
ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA;
GN; ML; MR; NE; SN; TD; TG

12/3,AB/17 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 1999 American Chemical Society. All rts. reserv.

128124228 CA: 128(11)124228m JOURNAL
Tagging genes and trapping promoters in Toxoplasma gondii by insertional mutagenesis
AUTHOR(S): Roos, David S.; Sullivan, William J.; Striepen, Boris; Bohne, Wolfgang; Donald, Robert G. K.
LOCATION: Department of Biology, University of Pennsylvania, Philadelphia
, PA, 19104-6018, USA
JOURNAL: Methods (Orlando, Fla.) DATE: 1997 VOLUME: 13 NUMBER: 2
PAGES: 112-122 CODEN: MTHDE9 ISSN: 1046-2023 LANGUAGE: English
PUBLISHER: Academic Press
? ds

Set	Items	Description
S1	5329	DHFR
S2	4603	RECOMBINASE??
S3	0	S1 AND S2
S4	11756	HOMOLOGOUS(W)RECOMBINATION
S5	38	S1 AND S4
S6	248	S2 AND S4
S7	278714	MARKER
S8	292	S4(10N)S7
S9	20	S2 AND S8
S10	58	S5 OR S9
S11	32	RD (unique items)
S12	17	S10 AND PY>=1997

? s transcription(w)factor??

Processing
434521 TRANSCRIPTION
3297718 FACTOR??
S13 113725 TRANSCRIPTION(W)FACTOR??
? s zinc(w)finger??

385393 ZINC
81273 FINGER??
S14 14247 ZINC(W)FINGER??
? s s13 or s14

113725 S13
14247 S14
S15 122696 S13 OR S14
? s s4 and s15

11756 S4
122696 S15
S16 331 S4 AND S15
? s s4(10n)s15

11756 S4
122696 S15
S17 8 S4(10N)S15
? s s4(20n)s15

11756 S4
122696 S15
S18 28 S4(20N)S15
? rd

...completed examining records
S19 15 RD (unique items)
? s s12 or s19

17 S12
15 S19
S20 32 S12 OR S19
? t s20/3,ab/1-32

20/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11489906 BIOSIS NO.: 199800271238
DNA cassette exchange in ES cells mediated by FLP %%%recombinase%%%: An
efficient strategy for repeated modification of tagged loci by marker-free constructs.

AUTHOR: Seibler Jost; Schuebeler Dirk; Fiering Steven; Groudine Mark; Bode
Juergen(a)
AUTHOR ADDRESS: (a)GBF, Natl. Res. Cent. Biotechnol., Mascheroder Weg 1,
D-38124 Braunschweig, Germany

JOURNAL: Biochemistry 37 (18):p6229-6234 May 5, 1998
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The repeated modification of a genomic locus is a technically demanding but powerful strategy to analyze the function of a particular gene product or the role of cis-regulatory DNA elements in mammalian cells. The initial step is "tagging" a site with a selectable %%%marker%%% which is done by %%%homologous%%% %%%recombination%%% (HR)
to modify a known locus or by random integration to study cis-regulatory elements at a reproducibly accessible genomic location. The tag is then used to target the construct of choice during an exchange step. Presented here is a novel technique in which the exchange is independent of HR and does not introduce vector sequences. It relies on our previous studies on the replacement of DNA cassettes by FLP-%%%recombinase%%%, whereby some common limitations can be overcome. To this end, the tag, a hygtk positive/negative selection marker, is integrated into the genome of embryonic stem (ES) cells. This marker is flanked by a wild-type Flp-recognition target (FRT) site on one end and by a modified heterospecific FRT site on the other. Successful Flp-mediated replacement of the hygtk cassette is enriched by ganciclovir (GANC) selection for cells that lack the encoded fusion protein. Thereby, the hygtk gene can be exchanged for virtually any sequence in a single efficient step without the need of introducing a positive selectable marker. The system can hence be used to analyze the function of either a gene product or regulatory sequences in ES cells or the transgenic mice derived thereof.

20/3,AB/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11295630 BIOSIS NO.: 199800076962
Transfection of malaria parasites.

AUTHOR: Waters A P(a); Thomas A W; Van Dijk M R(a); Janse C J(a)
AUTHOR ADDRESS: (a)Dep. Parasitol., Univ. Leiden, Postbox 9605, 2300 RC
Leiden, Netherlands

JOURNAL: Methods (Orlando) 13 (2):p134-147 Oct., 1997
ISSN: 1046-2023
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The stable genetic transformation of three phylogenetically diverse species of Plasmodium, the parasitic etiological agent of malaria, is now possible. The parasite is haploid throughout the vast majority of its life cycle. Therefore with the single selectable marker activity and protocols currently available, it is possible not only to express introduced transgenes but also to study the effects of site-specific %%%homologous%%% %%%recombination%%% such as gene knockout.
Transgene expression will allow the detailed study of many aspects of the cellular biology of malaria parasites, for example, the mechanisms underlying drug resistance and protein trafficking. We describe here the methods for propagation of the two animal models (Plasmodium berghei and Plasmodium knowlesi) and for transfection of these two species and the human parasite, Plasmodium falciparum. Examples of transgene expression are given.

20/3,AB/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11295628 BIOSIS NO.: 199800076960
Tagging genes and trapping promoters in Toxoplasma gondii by insertional mutagenesis.

AUTHOR: Roos David S(a); Sullivan William J; Striepen Boris; Bohne Wolfgang
; Donald Robert G K
AUTHOR ADDRESS: (a)Dep. Biol., Univ., Pennsylvania, 415 S. University Ave.,
Philadelphia, PA 19104-6018, USA

JOURNAL: Methods (Orlando) 13 (2):p112-122 Oct., 1997
ISSN: 1046-2023
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Plasmid vectors that incorporate sequence elements from the dehydrofolate reductase-thymidylate synthase (%%%DHFR%%%-TS) locus of
Toxoplasma gondii integrate into the parasite genome with remarkably high frequency (>1% of transfected parasites). These vectors may-but need not-include mutant %%%DHFR%%%-TS alleles that confer pyrimethamine resistance to transgenic parasites. Large genomic constructs integrate at the endogenous locus by %%%homologous%%% %%%recombination%%%, but
cDNA-derived sequences lacking long stretches of contiguous genomic DNA (due to intron excision) typically integrate into chromosomal DNA by nonhomologous recombination. Nonhomologous integration occurs effectively at random; and coupled with the high frequency of transformation, this allows a large fraction of the parasite genome to be tagged in a single electroporation cuvette. Genomic tagging permits insertional mutagenesis studies conceptually analogous to transposon mutagenesis in bacteria, yeast, Drosophila, etc. In theory (and, thus far, in practice), this allows identification of any gene whose inactivation is not lethal to the

haploid tachyzoite form of T. gondii and for which a suitable selection or screen is available. Transformation vectors can be engineered to facilitate rescue of the tagged locus and to include a variety of reporters or selectable markers. Genetic strategies are also possible, using reporters whose function can be assayed by metabolic, visual, or immunological screens to "trap" genes that are activated (or inactivated) under various conditions of interest.

20/3,AB/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11192574 BIOSIS NO.: 199799813719
Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene.

AUTHOR: Shimano Hitoshi Iichiro Shimomura; Hammer Robert E; Herz Joachim;
Goldstein Joseph L(a); Brown Michael S; Horton Jay D
AUTHOR ADDRESS: (a)Dep. Molecular Genetics, Univ. Texas Southwestern Med.
Cent., 5323 Harry Hines Blvd., Room L5-23, USA

JOURNAL: Journal of Clinical Investigation 100 (8):p2115-2124 1997
ISSN: 0021-9738
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The synthesis of cholesterol and its uptake from plasma LDL are regulated by two membrane-bound %%%transcription%%% %%%factors%%%, designated sterol regulatory element binding protein-1 and -2 (SREBP-1 and SREBP-2). Here, we used the technique of %%%homologous%%% %%%recombination%%% to generate mice with disruptions in the gene encoding the two isoforms of SREBP-1, termed SREBP-1a and SREBP-1c. Heterozygous gene-disrupted mice were phenotypically normal, but 5085% of the homozygous (-/-) mice died in utero at embryonic day 11. The surviving -/- mice appeared normal at birth and throughout life. Their livers expressed no functional SREBP-1. There was a 1.5-fold upregulation of SREBP-2 at the level of mRNA and a two- to threefold increase in the amount of mature SREBP-2 in liver nuclei. Previous studies showed that SREBP-2 is much more potent than SREBP-1c, the predominant hepatic isoform of SREBP-1, in activating transcription of genes encoding enzymes of cholesterol synthesis. Consistent with this observation, the SREBP- 1 -/- animals manifested elevated levels of mRNAs for 3-hydroxy-3-methylglutaryl coenzyme A synthase and reductase, farnesyl diphosphate synthase, and squalene synthase. Cholesterol synthesis, as measured by the incorporation of (3H)water, was elevated threefold in livers of the -/- mice, and hepatic cholesterol content was increased by 50%. Fatty acid synthesis was decreased in livers of the -/- mice. The amount of white adipose tissue was not significantly decreased, and the levels of mRNAs for lipogenic enzymes, adipocyte lipid binding protein, lipoprotein lipase, and leptin were normal in the -/- mice. We conclude from these studies that SREBP-2 can replace SREBP-1 in regulating cholesterol synthesis in livers of mice and that the higher potency of SREBP-2 relative to SREBP-1c leads to excessive hepatic cholesterol synthesis in these animals.

20/3,AB/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11131846 BIOSIS NO.: 199799752991
Homologous recombination based modification in Escherichia coli and germline transmission in transgenic mice of a bacterial artificial chromosome.

AUTHOR: Yang Xiangdong W; Model Peter; Heintz Nathaniel(a)
AUTHOR ADDRESS: (a)Lab. Molecular Biol., Box 260, Rockefeller Univ., 1230
York Ave., New York, NY 10021, USA

JOURNAL: Nature Biotechnology 15 (9):p859-865 1997
ISSN: 1087-0156
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Escherichia coli-based artificial chromosomes have become important tools for physical mapping and sequencing in various genome projects. The lack of a general method to modify these large bacterial clones, however, has limited their utility in functional studies. We developed a simple method to modify bacterial artificial chromosomes directly in the recombination-deficient E. coli host strain by %%%homologous%%% %%%recombination%%% for in vivo studies. The IRES-LacZ marker gene was introduced into a 131 kb SAC containing the murine %%%zinc%%% %%%finger%%% gene, RU49. No rearrangements or deletions were detected in the modified BACs. Furthermore, transgenic mice were generated by pronuclear injection of the modified SAC, and germline transmission of the intact SAC has been obtained. Proper expression of the lacZ transgene in the brain has been observed, which could not be obtained with conventional transgenic constructs.

20/3,AB/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10932707 BIOSIS NO.: 199799553852
Transfection of the primate malaria parasite Plasmodium knowlesi using entirely heterologous constructs.

AUTHOR: Van Der Wel Anna M Tomas; Kocken Clemens H M; Malhotra Pawan; Janse
Chris J; Waters Andrew P; Thomas Alan W(a)
AUTHOR ADDRESS: (a)Dep. Parastiol., Biomed. Primate Research, Centre, Lange
Kleiweg 151, 2288 GJ Rijwijk, Netherlands

JOURNAL: Journal of Experimental Medicine 185 (8):p1499-1503 1997
ISSN: 0022-1007
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The recently developed transfection systems for Plasmodium berghei and Plasmodium falciparum offer important new tools enabling further insight into the biology of malaria parasites. These systems rely upon artificial parasite-host combinations which do not allow investigation into the complex interactions between parasites and their natural hosts. Here we report on stable transfection of Plasmodium knowlesi (a primate malaria parasite that clusters phylogenetically with P. vivax) for which both natural and artificial experimental hosts are available. Transfection of this parasite offers the opportunity to further analyze the biology of antigens not only in a natural host but also in hosts that are closely related to humans. To facilitate future development of integration-dependent transfection in P. knowlesi, completely heterologous plasmids that would reduce %%%homologous%%% %%%recombination%%% at unwanted sites in the genome were constructed. These plasmids contained the pyrimethamine-resistant form of dihydrofolate reductase-thymidylate synthase (%%%dhfr%%%-ts) from Toxoplasma gondii or P. berghei, under control of either (a) P. berghei or (b) P. falciparum promoters. Plasmids were electroporated into mature P. knowlesi schizonts and these cells were injected into rhesus monkeys (Macaca mulatta). After pyrimethamine treatment of these monkeys, resistant parasites were obtained that contained the plasmids. Promoter regions of both P. berghei and P. falciparum controlling %%%dhfr%%%-ts expression were effective in conferring pyrimethamine resistance in P. knowlesi, indicating that common signals control gene expression in phylogenetically distant Plasmodium species.

20/3,AB/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10668956 BIOSIS NO.: 199799290101
Animal models for studying the genetic basis of metabolic regulation.

AUTHOR: Nizielski Steven E; Lechner Pamela S; Croniger Colleen M; Wang Nai-Dy; Darlington Gretchen J; Hanson Richard W(a)
AUTHOR ADDRESS: (a)Dep. Biochem., Case Western Reserve Univ. Sch. Med.,
Clevelsand, OH 44106-3649, USA

JOURNAL: Journal of Nutrition 126 (11):p2697-2708 1996

ISSN: 0022-3166
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Modern genetics has developed methods to modify the expression of genes in animals to study the factors responsible for the tissue-specific expression and hormonal and dietary regulation of metabolic processes. As these methods are applied to genes that code for critical proteins in metabolic pathways, a new insight into the control of metabolism is emerging. There are three general approaches currently in use. First, is the introduction of genes into the germ line to create transgenic animal models in which the gene of interest is over-expressed. This model is of particular value for promoter analysis because it is possible to introduce specific mutations into a putative regulatory region of a transgene and study its transcriptional control in the intact animal. Second, the developmental function of a gene product and its effect on various metabolic processes in a mouse can be directly determined by deleting a gene of interest by %%%homologous%%% %%%recombination%%%. Gene "knockout" mice are currently available with deletions in the genes for a variety of %%%transcription%%% %%%factors%%% and other biologically active proteins, permitting a critical analysis of the proteins responsible for the metabolic patterning of the animal. Third, the metabolic role of a gene of interest in a specific tissue can be studied by ablating its mRNA by the introduction of a transgene that codes for antisense mRNA targeted against the gene transcript. Because it is possible to use a transgene with a tissue-specific promoter, this procedure allows the isolation of the metabolic effect to a selected tissue in the transgenic animal. Taken together, these procedures provide a unique set of metabolic models for an in-depth study of metabolic regulation. This review will present examples of selected animal models currently available and will outline the challenge these animals present for investigators in the nutritional sciences.

20/3,AB/8 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09622812 BIOSIS NO.: 199598077730
Inducible expression of a neo gene integrated into the human alpha-globin gene cluster.

AUTHOR: Bernet-Grandaud Agnes(a); Ouazana Roland; Morle Francois; Godet Jacqueline
AUTHOR ADDRESS: (a)CNRS UMR 106, Univ. Claude-Bernard, Lyon-I, 43 Boulevard 11-Novembre-1918, 69622 Villeurbanne Ce, France

JOURNAL: Comptes Rendus de l'Academie des Sciences Serie III Sciences de la Vie 317 (10):p921-929 1994
ISSN: 0764-4469
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English; French
SUMMARY LANGUAGE: English; French

ABSTRACT: We replaced the 3' flanking region of the human alpha-1-globin gene that binds in vitro the specific %%%transcription%%% %%%factors%%% GATA-1 and API/NF-E2, by a neo marker gene using %%%homologous%%% %%%recombination%%% in a MEL (mouse erythroleukemia line) hybrid cell line harbouring a single human chromosome 16. Using an improved method of the neo-positive and HSV-tk negative selection, one correctly targeted clone was obtained out of 164 clones analyzed. In contrast to non-targeted clones, the expression of the neo gene in the targeted clone acquired the erythroid differentiation-dependent inducibility normally characteristic of the alpha-globin genes. No difference was observed in the expression of the human zeta, alpha-2, alpha-1, or theta-globin genes before and after induction of differentiation between the targeted clone and parental cells. These results indicate that, at least in the experimental system used the 3' flanking region of the human alpha-1-globin gene can be replaced by an exogenous non-erythroid gene without affecting the regulation of the globin genes contained in the alpha-globin cluster.

20/3,AB/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09304319 BIOSIS NO.: 199497312689
Dicistronic targeting constructs: Reporters and modifiers of mammalian gene expression.

AUTHOR: Mountford Peter(a); Zevnik Branko; Duewel Annette; Nichols Jennifer ; Li Meng; Dani Christian; Robertson Morag; Chambers Ian; Smith Austin
AUTHOR ADDRESS: (a)Cent. Anim. Biotechnol., Sch. Vet. Sci., Univ. Melb., Melbourne, Australia

JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 91 (10):p4303-4307 1994
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To investigate the activity of candidate regulatory molecules in mammalian embryogenesis, we have developed a general strategy for modifying and reporting resident chromosomal gene expression. The picornaviral internal ribosome-entry site was incorporated into gene targeting constructs to provide cap-independent translation of a selectable marker from fusion transcripts generated following %%%homologous%%% %%%recombination%%%. These promoterless constructs were highly efficient and have been used both to inactivate the stem-cell-specific %%%transcription%%% %%%factor%%% Oct-4 and to introduce a quantitative regulatory modification into the gene for a stem-cell maintenance factor, differentiation-inhibiting activity. In addition, the inclusion of a beta-galactosidase reporter gene in the constructs enabled accurate and sensitive detection of cellular sites of transcription. This has allowed visualization of putative "stem-cell niches" in which sources of elevated expression of differentiation-inhibiting activity were localized to the differentiated cells surrounding colonies of stem cells.

20/3,AB/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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09182035 BIOSIS NO.: 199497190405
Cloning and characterization of the G-box binding factor, an essential component of the developmental switch between early and late development in Dictyostelium.

AUTHOR: Schnitzler Gavin R; Fischer Wolfgang H; Firtel Richard A(a)
AUTHOR ADDRESS: (a)Dep. Biol., Cent. Mol. Genet., Univ. Calif., San Diego, La Jolla, CA 92093-0634, USA

JOURNAL: Genes & Development 8 (4):p502-514 1994
ISSN: 0890-9369
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: During Dictyostelium development, the cAMP-regulated induction of cell-type-specific late genes marks a developmental switch from the initial formation of the multicellular organism to the differentiation of the various cell types that mediate morphogenesis and eventually give rise to the mature fruiting body. The G-box binding factor (GBF) is a developmentally regulated Dictyostelium transcription factor whose affinity for a DNA sequence correlates with the ability of that sequence to confer inducibility to late gene promoters in response to high, continuous levels of extracellular cAMP. We report the purification of GBF and cloning of the gene that encodes it, as confirmed by in vitro production of GBF activity. The predicted protein is highly basic and contains two putative %%%zinc%%% %%%fingers%%%. Disruption of the

GBF
gene by %%%homologous%%% %%%recombination%%% results in the loss of all
GBF DNA-binding activity, developmental arrest at the loose aggregate stage, and the loss of late gene induction during development or in response to extracellular cAMP. Constitutive expression of GBF complements the null phenotype and allows for the rapid activation of a class of late genes in response to cAMP. Our results indicate that GBF acts as an extracellular cAMP-responsive transcriptional activator regulating late gene expression and is an essential component of a developmental switch between aggregation and cellular morphogenesis.

20/3,AB/11 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09036778 BIOSIS NO.: 199497045148
Mammalian achaete-scute homolog 1 is required for the early development of olfactory and autonomic neurons.

AUTHOR: Guillemot Francois(a); Lo Li-Ching; Johnson Jane E; Auerbach Anna
(a); Anderson David J; Joyner Alexandra L(a)
AUTHOR ADDRESS: (a)Samuel Lunenfeld Res. Inst., Mount Sinai Hosp., Toronto
M5G 1X5, Canada

JOURNAL: Cell 75 (3):p463-476 1993
ISSN: 0092-8674
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The mouse Mash-1 gene, like its Drosophila homologs of the achaete-scute complex (AS-C), encodes a %%%transcription%%% %%%factor%%%
expressed in neural precursors. We created a null allele of this gene by %%%homologous%%% %%%recombination%%% in embryonic stem cells. Mice
homozygous for the mutation die at birth with apparent breathing and feeding defects. The brain and spinal cord of the mutants appear normal, but their olfactory epithelium and sympathetic, parasympathetic, and enteric ganglia are severely affected. In the olfactory epithelium, neuronal progenitors die at an early stage, whereas the nonneuronal supporting cells are present. In sympathetic ganglia, the mutation arrests the development of neuronal precursors, preventing the generation of sympathetic neurons, but does not affect glial precursor cells. These observations suggest that Mash-1, like its Drosophila homologs of the AS-C, controls a basic operation in development of neuronal progenitors in distinct neural lineages.

20/3,AB/12 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07420717 BIOSIS NO.: 000091026706
REPLICATION OF AN ADENOVIRUS TYPE 34 MUTANT DNA CONTAINING TANDEM
REITERATIONS OF THE INVERTED TERMINAL REPEAT

AUTHOR: CHEN M; HORWITZ M S
AUTHOR ADDRESS: DEP. MICROBIOL.-IMMUNOL., ALBERT EINSTEIN COLL. OF MED.,
BRONX, NEW YORK 10461.

JOURNAL: VIROLOGY 179 (2). 1990. 567-575.
FULL JOURNAL NAME: Virology
CODEN: VIRLA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A mutant of human adenovirus type 34 (Ad34) has been isolated which contains DNA molecules with tandem reiterations of from two to eight copies of a 131-bp sequence within the right-sided inverted terminal repetition. Terminal heterogeneity was not eliminated by repeated plaque purifications indicating that the population of DNA molecules with various numbers of reiterations could rapidly evolve from the DNA of a single virus particle. These enlarged DNA molecules were capable of replication both in vivo and in vitro. The nucleotide sequence of the mutant Ad34 inverted-terminal repetitions contained most of the essential features of the Ad origin of DNA replication. These features include the ATAATATACC sequence which is present between the highly conserved bases 9-18 in all human adenoviruses, as well as the consensus sequences for the binding of nuclear factor I and nuclear factor III. However, the reiterated sequences lacked a dG appropriately placed on the template strand to serve as a potential site for internal initiation. It appears that the rapid amplification of two to eight copies of the reiterated terminal sequences does not arise from internal initiation during replication but probably from homologous recombination.

20/3,AB/13 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

07267544 EMBASE No: 1998156315
DNA cassette exchange in ES cells mediated by FLF %%%recombinase%%%: An
efficient strategy for repeated modification of tagged loci by marker-free constructs

Seibler J.; Schubeler D.; Fiering S.; Groudine M.; Bode J.
J. Bode, GBF, Natl. Res. Center for Biotechnology, Dartmouth Medical School, Mascheroder Weg 1, D-38124 Braunschweig Germany
Biochemistry (BIOCHEMISTRY) (United States) 05 MAY 1998, 37/18 (6229-6234)

CODEN: BICHA ISSN: 0006-2960
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 25

The repeated modification of a genomic locus is a technically demanding but powerful strategy to analyze the function of a particular gene product or the role of cis-regulatory DNA elements in mammalian cells. The initial step is 'tagging' a site with a selectable %%%marker%%% which is done by %%%homologous%%% %%%recombination%%% (HR) to modify a known locus or by
random integration to study cis-regulatory elements at a reproducibly accessible genomic location. The tag is then used to target the construct of choice during an exchange step. Presented here is a novel technique in which the exchange is independent of HR and does not introduce vector sequences. It relies on our previous studies on the replacement of DNA. cassettes by FLP-%%%recombinase%%%, whereby some common limitations can be
overcome. To this end, the tag, a hygtk positive/negative selection marker, is integrated into the genome of embryonic stem (ES) cells. This marker is flanked by a wild-type Flp-recognition target (FRT) site on one end and by a modified heterospecific FRT site on the other. Successful Flp-mediated replacement of the hygtk cassette is enriched by ganciclovir (GANC) selection for cells that lack the encoded fusion protein. Thereby, the hygtk gene can be exchanged for virtually any sequence in a single efficient step without the need of introducing a positive selectable marker. The system can hence be used to analyze the function of either a gene product or regulatory sequences in ES cells or the transgenic mice derived thereof.

20/3,AB/14 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

07133509 EMBASE No: 1998022357
Tagging genes and trapping promoters in Toxoplasma gondii by insertional mutagenesis

Roos D.S.; Sullivan W.J.; Striepen B.; Bohne W.; Donald R.G.E.
D.S. Roos, Department of Biology, University of Pennsylvania, 415 South University Avenue, Philadelphia, PA 19104-6018 United States
AUTHOR EMAIL: droos@sas.upenn.edu
Methods: A Companion to Methods in Enzymology (METHODS COMPANION METHODS
ENZYMOL.) (United States) 1997, 13/2 (112-122)

CODEN: MTHDE ISSN: 1046-2023
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 30

Plasmid vectors that incorporate sequence elements from the dehydrofolate reductase-thymidylate synthase (%%%DHFR%%%-TS) locus of Toxoplasma gondii
integrate into the parasite genome with remarkably high frequency (> 1% of transfected parasites). These vectors may - but need not - include mutant %%%DHFR%%%-TS alleles that confer pyrimethamine resistance to transgenic
parsites. Large genomic constructs integrate at the endogenous locus by %%%homologous%%% %%%recombination%%%, but cDNA-derived sequences lacking
long stretches of contiguous genomic DNA (due to intron excision) typically integrate into chromosomal DNA by nonhomologous recombination. Nonhomologous integration occurs effectively at random; and coupled with the high frequency of transformation, this allows a large fraction of the parasite genome to be tagged in a single electroporation cuvette. Genomic tagging permits insertional mutagenesis studies conceptually analogous to transposon mutagenesis in bacteria, yeast, Drosophila, etc. In theory (and, thus far, in practice), this allows identification of any gene whose inactivation is not lethal to the haploid tachyzoite form of T. gondii and for which a suitable selection or screen is available. Transformation vectors can be engineered to facilitate rescue of the tagged locus and to include a variety of reporters or selectable markers. Genetic strategies are also possible, using reporters whose function can be assayed by metabolic, visual, or immunological screens to 'trap' genes that are activated (or inactivated) under various conditions of interest.

20/3,AB/15 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE
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06995773 EMBASE No: 1997281953
Homologous recombination based modification in Escherichia coli and germline transmission in transgenic mice of a bacterial artificial chromsome
Yang X.W.; Model P.; Heintz N.
N. Heintz, Laboratory of Molecular Biology, Howard Hughes Med. Med. Institute, Rockefeller University, 1230 York Ave., New York, NY 10021 United States
Nature Biotechnology (NAT. BIOTECHNOL.) (United States) 1997, 15/9 (859-865)

CODEN: NABIF ISSN: 0733-222X
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 34

Escherichia coli-based artificial chromosomes have become important tools for physical mapping and sequencing in various genome projects. The lack of a general method to modify these large bacterial clones, however, has limited their utility in functional studies. We developed a simple method to modify bacterial artificial chromosomes directly in the recombination-deficient E. coli host strain by %%%homologous%%% %%%recombination%%% for
in viva studies. The IRES-LacZ marker gene was introduced into a 131 kb BAC
containing the murine %%%zinc%%% %%%finger%%% gene, RU49. No rearrangements
or deletions were detected in the modified BACs. Furthermore, transgenic mice were generated by pronuclear injection of the modified BAC, and germline transmission of the intact BAC has been obtained. Proper expression of the lacZ transgene in the brain has been observed, which could not be obtained with conventional transgenic constructs.

20/3,AB/16 (Item 4 from file: 73)
DIALOG(R)File 73:EMBASE
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06849190 EMBASE No: 1997131778
Transfection of the primate malaria parasite Plasmodium knowlesi using entirely heterologous constructs
Van der Wel A.M.; Tomas A.M.; Kocken C.H.M.; Malhotra P.; Janse C.J.; Waters A.P.; Thomas A.W.
A.W. Thomas, Department of Parasitology, Biomedical Primate Research Centre, Lange Kleiweg 151, 2288 GJ Rijswijk Netherlands
Journal of Experimental Medicine (J. EXP. MED.) (United States) 1997, 185/8 (1499-1503)

CODEN: JEMEA ISSN: 0022-1007
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 23

The recently developed transfection systems for Plasmodium berghei and Plasmodium falciparum offer important new tools enabling further insight into the biology of malaria parasites. These systems rely upon artificial parasite-host combinations which do not allow investigation into the complex interactions between parasites and their natural hosts. Here we report on stable transfection of Plasmodium knowlesi (a primate malaria parasite that cluster phylogenetically with P. vivax) for which both natural and artificial experimental hosts are available. Transfection of this parasite offers the opportunity to further analyze the biology of antigens not only in a natural host but also in hosts that are closely related to humans. To facilitate future development of integration-dependent transfection in P. knowlesi, completely heterologous plasmids that would reduce %%%homologous%%% %%%recombination%%% at unwanted
sites in the genome were constructed. These plasmids contained the pyrimethamine-resistant form of dihydrofolate reductase-thymidylate synthase (%%%dhfr%%%-ts) from Toxoplasma gondii or P. berghei, under control of either (a) P. berghei or (b) P. falciparum promoters. Plasmids were electroporated into mature P. knowlesi schizonts and these cells were injected into rhesus monkeys (Macaca mulatta). After pyrimethamine treatment of these monkeys, resistant parasites were obtained that continued the plasmids. Promoter regions of both P. berghei and P. falciparum controlling %%%dhfr%%%-ts expression were effective in conferring pyrimethamine resistance in P. knowlesi, indicating that common signals control gene expression in phylogenetically distant Plasmodium species.

20/3,AB/17 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1999 Dialog Corporation. All rts. reserv.

09878061 99067845
Thymocyte selection in Vav and IRF-1 gene-deficient mice.
Penninger JM; Mak TW
Amgen Institute, Department of Medical Biophysics, University of Toronto, Ontario, Canada. Jpenning@amgen.com
Immunol Rev (DENMARK) Oct 1998, 165 p149-66, ISSN 0105-2896 Journal Code: GG4
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, ACADEMIC
T cells undergo a defined program of phenotypic and genetic changes during differentiation within the thymus. These changes define commitment of T-cell receptor (TCR) gamma delta and TCR alpha beta cells and lineage differentiation into CD4+ T helper and CD8+ cytotoxic T cells. T-cell differentiation and selection in the thymus constitute a tightly co-ordinated multistep journey through a network that can be envisaged as a three-dimensional informational highway made up of stromal cells and extracellular matrix molecules. This intrathymic journey is controlled by information exchange, with thymocytes depending on two-way cellular interactions with thymic stromal cells in order to receive essential signals for maturation and selection. Genetic inactivation of surface receptors, signal transduction molecules, and %%%transcription%%% %%%factors%%% using %%%homologous%%% %%%recombination%%% has provided novel
insight into the signaling cascades that relay surface receptor engagement to gene transcription and subsequent progression of the developmental program. In this review we discuss molecular mechanisms of T lymphocyte development in mice that harbour genetic mutations in the guanine nucleotide exchange factor Vav and the interferon regulatory transcription factor 1 (IRF-1). We also propose a novel model of T-cell selection based on TCR alpha chain-directed signals for allelic exclusion and TCR alpha-based selection for single receptor usage.

20/3,AB/18 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1999 Dialog Corporation. All rts. reserv.

09517827 98241343
DNA cassette exchange in ES cells mediated by Flp %%%recombinase%%%: an
efficient strategy for repeated modification of tagged loci by marker-free constructs.

Seibler J; Schubeler D; Fiering S; Groudine M; Bode J
GBF, National Research Center for Biotechnology, Mascheroder Weg 1, D-38124 Braunschweig.
Biochemistry (UNITED STATES) May 5 %%%1998%%%, 37 (18) p6229-34,
ISSN 0006-2960 Journal Code: A0G
Contract/Grant No.: CA54337, CA, NCI; DK44746, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The repeated modification of a genomic locus is a technically demanding but powerful strategy to analyze the function of a particular gene product or the role of cis-regulatory DNA elements in mammalian cells. The initial step is "tagging" a site with a selectable %%%marker%%% which is done by %%%homologous%%% %%%recombination%%% (HR) to modify a known locus or by
random integration to study cis-regulatory elements at a reproducibly accessible genomic location. The tag is then used to target the construct of choice during an exchange step. Presented here is a novel technique in which the exchange is independent of HR and does not introduce vector sequences. It relies on our previous studies on the replacement of DNA cassettes by FLP-%%%recombinase%%%, whereby some common limitations can be
overcome. To this end, the tag, a hygtk positive/negative selection marker, is integrated into the genome of embryonic stem (ES) cells. This marker is flanked by a wild-type Flp-recognition target (FRT) site on one end and by a modified heterospecific FRT site on the other. Successful Flp-mediated replacement of the hygtk cassette is enriched by ganciclovir (GANC) selection for cells that lack the encoded fusion protein. Thereby, the hygtk gene can be exchanged for virtually any sequence in a single efficient step without the need of introducing a positive selectable marker. The system can hence be used to analyze the function of either a gene product or regulatory sequences in ES cells or the transgenic mice derived thereof.

20/3,AB/19 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1999 Dialog Corporation. All rts. reserv.

09354238 98071021
Tagging genes and trapping promoters in Toxoplasma gondii by insertional mutagenesis.
Roos DS; Sullivan WJ; Striepen B; Bohne W; Donald RG
Department of Biology, University of Pennsylvania, 415 South University Avenue, Philadelphia, Pennsylvania, 19104-6018, USA. droos@sas.upenn.edu
Methods (UNITED STATES) Oct %%%1997%%%, 13 (2) p112-22, ISSN
1046-2023 Journal Code: CPO
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Plasmid vectors that incorporate sequence elements from the dehydrofolate reductase-thymidylate synthase (%%%DHFR%%%-TS) locus of Toxoplasma gondii
integrate into the parasite genome with remarkably high frequency (>1% of transfected parasites). These vectors may-but need not-include mutant %%%DHFR%%% -TS alleles that confer pyrimethamine resistance to transgenic
parasites. Large genomic constructs integrate at the endogenous locus by %%%homologous%%% %%%recombination%%%, but cDNA-derived sequences lacking
long stretches of contiguous genomic DNA (due to intron excision) typically integrate into chromosomal DNA by nonhomologous recombination. Nonhomologous integration occurs effectively at random; and coupled with the high frequency of transformation, this allows a large fraction of the parasite genome to be tagged in a single electroporation cuvette. Genomic tagging permits insertional mutagenesis studies conceptually analogous to transposon mutagenesis in bacteria, yeast, Drosophila, etc. In theory (and, thus far, in practice), this allows identification of any gene whose inactivation is not lethal to the haploid tachyzoite form of T. gondii and for which a suitable selection or screen is available. Transformation vectors can be engineered to facilitate rescue of the tagged locus and to include a variety of reporters or selectable markers. Genetic strategies are also possible, using reporters whose function can be assayed by metabolic, visual, or immunological screens to "trap" genes that are activated (or inactivated) under various conditions of interest. Copyright 1997 Academic Press.

20/3,AB/20 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09043326 97272086
Transfection of the primate malaria parasite Plasmodium knowlesi using entirely heterologous constructs.
van der Wel AM; Tomas AM; Kocken CH; Malhotra P; Janse CJ; Waters AP;
Thomas AW
Department of Parasitology, Biomedical Primate Research Centre, Rijswijk, The Netherlands.
J Exp Med (UNITED STATES) Apr 21 %%%1997%%%, 185 (8) p1499-503, ISSN
0022-1007 Journal Code: I2V
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The recently developed transfection systems for Plasmodium berghei and Plasmodium falciparum offer important new tools enabling further insight into the biology of malaria parasites. These systems rely upon artificial parasite-host combinations which do not allow investigation into the complex interactions between parasites and their natural hosts. Here we report on stable transfection of Plasmodium knowlesi (a primate malaria parasite that clusters phylogenetically with P. vivax) for which both natural and artificial experimental hosts are available. Transfection of this parasite offers the opportunity to further analyze the biology of antigens not only in a natural host but also in hosts that are closely related to humans. To facilitate future development of integration-dependent transfection in P. knowlesi, completely heterologous plasmids that would reduce %%%homologous%%% %%%recombination%%% at unwanted
sites in the genome were constructed. These plasmids contained the pyrimethamine-resistant form of dihydrofolate reductase-thymidylate synthase (%%%dhfr%%% -ts) from Toxoplasma gondii or P. berghei, under
control of either (a) P. berghei or (b) P. falciparum promoters. Plasmids were electroporated into mature P. knowlesi schizonts and these cells were injected into rhesus monkeys (Macaca mulatta). After pyrimethamine treatment of these monkeys, resistant parasites were obtained that contained the plasmids. Promoter regions of both P. berghei and P. falciparum controlling %%%dhfr%%% -ts expression were effective in conferring pyrimethamine resistance in P. knowlesi, indicating that common signals control gene expression in phylogenetically distant Plasmodium species.

20/3,AB/21 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0221504 DBA Accession No.: 98-03101 PATENT
DNA construct for transforming yeast - containing a self-eliminating combination of a %%%recombinase%%% gene and a selectable marker
AUTHOR: Ashikari T; Kondo H; Sakakibara K; Araki H; Oshima Y
CORPORATE SOURCE: Osaka, Japan.
PATENT ASSIGNEE: Suntory %%%1997%%%
PATENT NUMBER: EP 814165 PATENT DATE: 971229 WPI ACCESSION NO.: 98-044341
(9805)
PRIORITY APPLIC. NO.: JP 96179820 APPLIC. DATE: 960621
NATIONAL APPLIC. NO.: EP 97304306 APPLIC. DATE: 970616
LANGUAGE: English
ABSTRACT: A new DNA construct (in the form of a plasmid) contains: a DNA
fragment capable of recombination with chromosomal DNA; a %%%recombinase%%%-sensitive (RS) sequence; a recombinant gene under the
control of an inducible promoter; a selectable marker; a second RS sequence; and a second DNA fragment capable of recombination with chromosomal DNA. The RS sequences are orientated in the same direction and each has a defined DNA sequence where the RS sequence nearest to the %%%recombinase%%% gene lacks 0-10 terminal nucleotides from the end
opposite to the %%%recombinase%%% gene and the RS sequence nearest to
the selectable marker lacks 0-10 terminal nucleotides from the end opposite to the selectable marker. The new DNA may be used for the transformation of yeast cells by: integrating the DNA into a yeast chromosome by recombination between the chromosomal DNA and the new DNA

fragments; selecting cells having integrated the DNA construct on the basis of expression of the selectable marker; and inducing the promoter to express the %%%recombinase%%% gene, causing recombination between the 1st and 2nd RS sequences and elimination of the %%%recombinase%%% gene, promoter and selectable marker. (39pp)

20/3,AB/22 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0219018 DBA Accession No.: 98-00615 PATENT
New genetic constructs for transformation of organisms, particularly plants - Cre-%%%recombinase%%% or Flp-%%%recombinase%%% co-expression with a ribozyme, antisense RNA, sense suppression RNA or plant growth factor biosynthetic gene, in a tobacco or potato transgenic plant
AUTHOR: Surin B P; de Feyter R C; Graham M W; Waterhouse P M; Keese P K ; Shahjahan A
CORPORATE SOURCE: Campbell, Australian Capital Territory, Australia; Acton, Australian Capital Territory, Australia.
PATENT ASSIGNEE: CSIRO; Univ.Australian-Nat. %%%1997%%%
PATENT NUMBER: WO 9737012 PATENT DATE: 971009 WPI ACCESSION NO.: 97-526087 (9748)
PRIORITY APPLIC. NO.: AU 969031 APPLIC. DATE: 960329
NATIONAL APPLIC. NO.: WO 97AU197 APPLIC. DATE: 970327
LANGUAGE: English
ABSTRACT: A new construct has a DNA cassette with a %%%recombinase%%% unit (with a Cre-%%%recombinase%%% or Flp-%%%recombinase%%% gene, terminator and 1st promoter) linked to a transgene unit (with 1 or more transgenes and 2nd promoters), flanked by 2 %%%recombinase%%% -binding recombination loci (e.g. lox or frt sites). The transgene may encode a ribozyme, antisense RNA, co-suppression RNA, or may be a selectable marker, reporter gene or an auxin or cytokinin biosynthetic gene or regulatory sequence (e.g. an ipt gene). An intron may be inserted to disrupt %%%recombinase%%% expression. The cassette may be expressed in a tobacco (Nicotiana tabacum), potato (Solanum tuberosum), sweet potato, Jerusalem artichoke, taro, yam, eucalyptus, pine, aspen, gerbera, chrysanthemum, orchid, lily, rose, fuchsia, azalea, carnation, camellia, gardenia, orange, lemon, grapefruit, tangerine, lime, apple, pear, strawberry, raspberry, loganberry, blackberry, sugarcane, banana, plantain, pineapple or asparagus transgenic plant. The construct may be used for selective removal or integration of transgenes, with tight regulation of expression. (84pp)

20/3,AB/23 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0216607 DBA Accession No.: 97-11728
Cre/loxP-mediated excision of a neomycin-resistance expression unit from an integrated retroviral vector increases long terminal repeat-driven transcription in human hematopoietic cells - DNA cassette excision by homologous recombination, for improved retro virus vector-mediated gene transfer and gene therapy
AUTHOR: Fernex C; Dubreuil P; Mannoni P; +Bagnis C
CORPORATE AFFILIATE: Inst.Paoli-Calmettes INSERM
CORPORATE SOURCE: Centre de Therapie Genique, Institut Paoli-Calmettes, 232 Boulevard de Sainte-Marguerite, BP 156, 13273 Marseilles Cedex 09, France. email:bagnisc@marseille.fnclcc.fr
JOURNAL: J.Virol. (71, 10, 7533-40) %%%1997%%%
ISSN: 0022-538X CODEN: JOVIAM
LANGUAGE: English
ABSTRACT: A Cre-loxP recombination system was developed for specific excision of a selectable marker expression unit from integrated retro viruses after gene transfer to hematopoietic cells, in order to increase target gene expression levels. A retro virus vector containing both a neomycin-resistance expression unit, flanked by loxP sites, and a granulocyte-macrophage colony stimulating factor (GM-CSF) cDNA was used to transduce a human hematopoietic K562 cell culture. Four transduced clones were then superinfected with a retro virus vector containing a Cre-%%%recombinase%%% expression unit. In 30 doubly transduced subclones, there was a strict correlation between cre expression and loxP-flanked DNA cassette excision, implying that Cre-%%%recombinase%%% activity was very efficient with retro virus vectors. Excision of the selectable marker cassette resulted in a significant increase in GM-CSF gene transcription, driven by the retro virus promoter. This system should be useful in gene therapy, and should reduce expression of undesirable additional foreign gene products. (37 ref)

20/3,AB/24 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0211797 DBA Accession No.: 97-06918
Transfection of the primate malaria parasite Plasmodium knowlesi using entirely heterologous constructs - for use in malaria recombinant vaccine development
AUTHOR: van der Wel A M; Tomas A M; Kocken C H M; Malhotra P; Janse C J ; Waters A P; +Thomas A W
CORPORATE AFFILIATE: Biomed.Primate-Res.Cent.Rijswijk Univ.Leiden Cent.Malaria-Other-Trop.Dis.Lisbon
CORPORATE SOURCE: Department of Parasitology, Biomedical Primate Research Centre, Lange Kleiweg 151, 2288 GJ Rijswijk, The Netherlands.
JOURNAL: J.Exp.Med. (185, 8, 1499-1503) %%%1997%%%
ISSN: 0022-1007 CODEN: JEMEAV
LANGUAGE: English
ABSTRACT: A system was developed for stable transfection of Plasmodium knowlesi (a primate malaria parasite in the Plasmodium vivax cluster). Completely heterologous vectors (plasmid pDT.Tg23 and plasmid pchD5.1/C3) designed to reduce %%%homologous%%% %%%recombination%%% at unwanted sites in the genome, were constructed. The plasmids contained the pyrimethamine resistant form of the dihydrofolate-reductase (EC-1.5.1.3)-thymidylate-synthase (EC-2.1.1.45) (%%%dhfr%%%-ts) gene from Toxoplasma gondii or Plasmodium berghei, under the control of a P. berghei or Plasmodium falciparum promoter. Plasmids were introduced into mature P. knowlesi schizonts by electroporation, and recombinant cells were injected into rhesus monkeys. After pyrimethamine treatment of monkeys, resistant parasites containing plasmids were obtained. Both the P. berghei and P. falciparum promoter regions controlling %%%dhfr%%% -ts expression conferred pyrimethamine resistance in P. knowlesi, indicating that common signals controlled gene expression in phylogenetically distant Plasmodium spp. This system should be useful in malaria recombinant vaccine production. (23 ref)

20/3,AB/25 (Item 5 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0210191 DBA Accession No.: 97-05312
Ligand-inducible gene targeting in mice - tamoxifen administration to chimeric Cre-%%%recombinase%%%-expressing transgenic mouse for tkneo selectable %%%marker%%% gene excision; %%%homologous%%% %%%recombination%%% (conference abstract)
AUTHOR: Feil R; Brocard J; Mascrez B; LeMeur M; Metzger D; Chambon P
CORPORATE AFFILIATE: CNRS INSERM Univ.Strasbourg-Louis-Pasteur
CORPORATE SOURCE: Institut de Genetique et de Biologie Moleculaire et Cellulaire, CNRS, INSERM, Universite Louis Pasteur, College de France, 67404 Illkirch-Cedex, C.U. de Strasbourg, France.
JOURNAL: Arch.Pharmacol. (355, 4, Suppl., 41) %%%1997%%%
ISSN: 0028-1298 CODEN: NSAPCC
CONFERENCE PROCEEDINGS: Deutsche Gesellschaft fuer Experimentelle und Klinische Pharmakologie und Toxikologie, 38th Spring Meeting, Mainz, Germany, 11-13 March, 1997.
LANGUAGE: English
ABSTRACT: Current gene targeting technology does not allow gene

inactivation at a given time and/or in a given tissue. To achieve conditional gene inactivation in mice, a ligand-inducible site-specific recombination system based on an engineered Cre-%%%recombinase%%% was set up. Cre was fused to a mutated ligand-binding domain of a human estrogen receptor supposed to bind the synthetic ligand tamoxifen but not endogenous estradiol. Transgenic mice were generated, expressing the chimeric %%%recombinase%%% (Cre-ERT) under the control of a cytomegalo virus promoter and were analyzed for the excision of a chromosomally-integrated tkneo gene flanked by loxP sites. Marker gene excision was induced by tamoxifen administration to the transgenic mice. This conditional site-specific recombination system should allow the analysis of knockout phenotypes that cannot be addressed by conventional gene targeting. (0 ref)

20/3,AB/26 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 1999 American Chemical Society. All rts. reserv.

128240335 CA: 128(20)240335t PATENT
Mammalian and human gene REC2 recombinases to induce transformation by homologous recombination and their promoters for sensitization of cells to irradiation
INVENTOR(AUTHOR): Holloman, William K.; Rice, Michael C.; Smith, Sheryl
T.; Shu, Zhigang; Kmiec, Eric B.
LOCATION: USA
ASSIGNEE: Thomas Jefferson University; Cornell Research Foundation, Inc.
PATENT: PCT International ; WO 9811214 A1 DATE: 19980319
APPLICATION: WO 97IB1217 (19970911) *US 25929 (19960911)
PAGES: 88 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-015/12A;
C12N-015/87B; C12P-019/34B DESIGNATED COUNTRIES: AL; AM; AU; AZ; BA; BB;
BG; BR; BY; CA; CN; CU; CZ; EE; GE; GH; HU; IL; IS; JP; KG; KP; KR; KZ; LC;
LK; LR; LT; LV; MD; MG; MK; MN; MX; NO; NZ; PL; RO; RU; SG; SI; SK; SL; TJ;
TM; TR; TT; UA; UZ; VN; YU; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM
DESIGNATED REGIONAL: GH; KE; LS; MW; SD; SZ; UG; ZW; AT; BE; CH; DE; DK;
ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA;
GN; ML; MR; NE; SN; TD; TG

20/3,AB/27 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
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128137142 CA: 128(12)137142d JOURNAL
Transcription factor Mts1/Mts2 (Atf1/Pcr1, Gad7/Pcr1) activates the M26 meiotic recombination hotspot in Schizosaccharomyces pombe
AUTHOR(S): Kon, Ming; Krawchuk, Michelle D.; Warren, B. Greg; Smith, Gerald R.; Wahls, Wayne P.
LOCATION: Dep. Biochemistry, Vanderbilt Univ. Sch. Med., Nashville, TN, 37232-0146, USA
JOURNAL: Proc. Natl. Acad. Sci. U. S. A. DATE: 1997 VOLUME: 94
NUMBER: 25 PAGES: 13765-13770 CODEN: PNASA6 ISSN: 0027-8424
LANGUAGE: English PUBLISHER: National Academy of Sciences

20/3,AB/28 (Item 3 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 1999 American Chemical Society. All rts. reserv.

128124228 CA: 128(11)124228m JOURNAL
Tagging genes and trapping promoters in Toxoplasma gondii by insertional mutagenesis
AUTHOR(S): Roos, David S.; Sullivan, William J.; Striepen, Boris; Bohne, Wolfgang; Donald, Robert G. K.
LOCATION: Department of Biology, University of Pennsylvania, Philadelphia
, PA, 19104-6018, USA
JOURNAL: Methods (Orlando, Fla.) DATE: 1997 VOLUME: 13 NUMBER: 2
PAGES: 112-122 CODEN: MTHDE9 ISSN: 1046-2023 LANGUAGE: English
PUBLISHER: Academic Press

20/3,AB/29 (Item 4 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 1999 American Chemical Society. All rts. reserv.

126326357 CA: 126(25)326357x JOURNAL
The winged helix transcription factor MFH1 is required for proliferation and patterning of paraxial mesoderm in the mouse embryo
AUTHOR(S): Winnier, Glenn E.; Hargett, Linda; Hogan, Brigid L. M.
LOCATION: Dep. Cell Biology Howard Hughes Medical Inst., Vanderbilt Univ.
Medical School, Nashville, TN, 37232-2175, USA
JOURNAL: Genes Dev. DATE: 1997 VOLUME: 11 NUMBER: 7 PAGES: 926-940
CODEN: GEDEEP ISSN: 0890-9369 LANGUAGE: English
PUBLISHER: Cold Spring
Harbor Laboratory Press

20/3,AB/30 (Item 5 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 1999 American Chemical Society. All rts. reserv.

126170497 CA: 126(13)170497e PATENT
Nucleic acid cassettes to select mutagenized microorganism strains with enhanced metabolism, metabolism-related enzymes and transcription factors, and fermentation
INVENTOR(AUTHOR): De Graaff, Leendert Hendrik; Van Den Broeck, Henriette
Catha; Visser, Jacob
LOCATION: Neth.
ASSIGNEE: Rijkslandbouwuniversiteit Wageningen; De Graaff, Leendert Hendrik; Van Den Broeck, Henriette Catharina; Visser, Jacob
PATENT: PCT International ; WO 9700962 A1 DATE: 19970109
APPLICATION: WO 96NL259 (19960624) *EP 95201707 (19950623) *EP 95202346
(19950830)
PAGES: 103 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-015/31A;
C12N-015/80B; C12N-015/65B; C12N-015/63B; C12N-015/10B; C12N-001/15B;
C07K-014/38B; C12Q-001/02B; C12Q-001/68B; C12N-001/15J; C12R-001/645J
DESIGNATED COUNTRIES: AL; AM; AT; AU; AZ; BB; BG; BR; BY; CA; CH; CN; CZ;
DE; DK; EE; ES; FI; GB; GE; HU; IL; IS; JP; KE; KG; KP; KR; KZ; LK; LR; LS;
LT; LU; LV; MD; MG; MK; MN; MW; MX; NO; NZ; PL; PT; RO; RU; SD; SE; SG
DESIGNATED REGIONAL: KE; LS; MW; SD; SZ; UG; AT; BE; CH; DE; DK; ES; FI;
FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; BF; BJ; CF; CG; CI; CM; GA

20/3,AB/31 (Item 6 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 1999 American Chemical Society. All rts. reserv.

124281029 CA: 124(21)281029g JOURNAL
Complexity of the erythroid transcription factor NF-E2 as revealed by gene targeting of the mouse p18 NF-E2 locus
AUTHOR(S): Kotkow, Karen J.; Orkin, Stuart H.
LOCATION: Division Hematology-Oncology, Children's Hospital, Boston, MA,
02115, USA
JOURNAL: Proc. Natl. Acad. Sci. U. S. A. DATE: 1996 VOLUME: 93
NUMBER: 8 PAGES: 3514-18 CODEN: PNASA6 ISSN: 0027-8424
LANGUAGE:
English

20/3,AB/32 (Item 7 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)
(c) 1999 American Chemical Society. All rts. reserv.

121127071 CA: 121(11)127071w PATENT
Activating expression of an amplifying endogenous gene by homologous

recombination
INVENTOR(AUTHOR): Treco, Douglas A.; Heartlein, Michael W.; Selden, Richard F.
LOCATION: USA
ASSIGNEE: Transkaryotic Therapies, Inc.
PATENT: PCT International ; WO 9412650 A2 DATE: 940609
APPLICATION: WO 93US11704 (931202) *US 985586 (921203)
PAGES: 93 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-015/90A;
A61K-048/00B; C12N-015/18B; C12N-015/27B DESIGNATED COUNTRIES: AU; CA; JP;
KR; NZ DESIGNATED REGIONAL: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LU
; MC; NL; PT; SE
? log

09/203500

Set Items Description

? s dhfr

S1 5229 DHFR

? s target or targetting or targeting or targets or targetted or targeted or integrate or integrates or integrating or integrated

336222 TARGET
1032 TARGETTING
71542 TARGETING
92366 TARGETS
746 TARGETTED
63376 TARGETED
14345 INTEGRATE
5508 INTEGRATES
15553 INTEGRATING
104624 INTEGRATED

S2 632685 TARGET OR TARGETTING OR TARGETING OR TARGETS OR TARGETTED
OR TARGETED OR INTEGRATE OR INTEGRATES OR INTEGRATING OR
INTEGRATED

? s homologous

S3 219836 HOMOLOGOUS

? s s1 and s2 and s3

5229 S1
632685 S2
219836 S3

S4 47 S1 AND S2 AND S3

? rd

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S5 23 RD (unique items)

? s s5 and py<1998

Processing

23 S5
28044780 PY<1998

S6 19 S5 AND PY<1998

? t s6/3,ab/1-19

6/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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11295628 BIOSIS NO.: 199800076960
Tagging genes and trapping promoters in Toxoplasma gondii by insertional mutagenesis.
AUTHOR: Roos David S(a); Sullivan William J; Striepen Boris; Bohne Wolfgang
; Donald Robert G K
AUTHOR ADDRESS: (a)Dep. Biol., Univ., Pennsylvania, 415 S. University Ave.,
Philadelphia, PA 19104-6018**USA
JOURNAL: Methods (Orlando) 13 (2):p112-122 Oct., 1997
ISSN: 1046-2023
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Plasmid vectors that incorporate sequence elements from the dehydrofolate reductase-thymidylate synthase (%%%DHFR%%%-TS) locus of
Toxoplasma gondii %%%integrate%%% into the parasite genome with remarkably high frequency (>1% of transfected parasites). These vectors may-but need not-include mutant %%%DHFR%%%-TS alleles that confer pyrimethamine resistance to transgenic parasites. Large genomic constructs %%%integrate%%% at the endogenous locus by %%%homologous%%%
recombination, but cDNA-derived sequences lacking long stretches of contiguous genomic DNA (due to intron excision) typically %%%integrate%%%
into chromosomal DNA by nonhomologous recombination. Nonhomologous integration occurs effectively at random; and coupled with the high frequency of transformation, this allows a large fraction of the parasite genome to be tagged in a single electroporation cuvette. Genomic tagging permits insertional mutagenesis studies conceptually analogous to transposon mutagenesis in bacteria, yeast, Drosophila, etc. In theory (and, thus far, in practice), this allows identification of any gene whose inactivation is not lethal to the haploid tachyzoite form of T. gondii and for which a suitable selection or screen is available. Transformation vectors can be engineered to facilitate rescue of the tagged locus and to include a variety of reporters or selectable markers. Genetic strategies are also possible, using reporters whose function can be assayed by metabolic, visual, or immunological screens to "trap" genes that are activated (or inactivated) under various conditions of interest.

6/3,AB/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10754744 BIOSIS NO.: 199799375889
In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition.
AUTHOR: Robinett Carmen C; Straight Aaron; Li Gang; Willhelm Carol; Sudlow
Gail; Murray Andrew; Belmont Andrew S(a)
AUTHOR ADDRESS: (a)Dep. Cell Structural Biol., B107 Chem. Life Sci. Lab.,
Univ. Ill. Urbana-Champaign, 601 S. Goodw**USA
JOURNAL: Journal of Cell Biology 135 (6 PART 2):p1685-1700 1996
ISSN: 0021-9525
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We report a new method for in situ localization of DNA sequences
that allows excellent preservation of nuclear and chromosomal ultrastructure and direct, in vivo observations. 256 direct repeats of the lac operator were added to vector constructs used for transfection and served as a tag for labeling by lac repressor. This system was first characterized by visualization of chromosome homogeneously staining regions (HSRs) produced by gene amplification using a dihydrofolate reductase (%%%DHFR%%%) expression vector with methotrexate selection.
Using electron microscopy, most HSRs showed apprx 100-nm fibers, as described previously for the bulk, large-scale chromatin organization in these cells, and by light microscopy, distinct, large-scale chromatin fibers could be traced in vivo up to 5 mu-m in length. Subsequent experiments demonstrated the potential for more general applications of this labeling technology. Single and multiple copies of the %%%integrated%%% vector could be detected in living CHO cells before gene
amplification, and detection of a single 256 lac operator repeat and its stability during mitosis was demonstrated by its %%%targeted%%% insertion
into budding yeast cells by %%%homologous%%% recombination. In both CHO
cells and yeast, use of the green fluorescent protein-lac repressor protein allowed extended, in vivo observations of the operator-tagged chromosomal DNA. Future applications of this technology should facilitate structural, functional, and genetic analysis of chromatin organization, chromosome dynamics, and nuclear architecture.

6/3,AB/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
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10236930 BIOSIS NO.: 199698691848
Transformation of Plasmodium falciparum malaria parasites by %%%homologous%%% integration of plasmids that confer resistance to pyrimethamine.
AUTHOR: Wu Yimin; Kirkman Laura A; Wellems Thomas E
AUTHOR ADDRESS: Lab. Parasitic Diseases, Natl. Inst. Allergy and Infectious Diseases, Natl. Inst. Health, Bethesda, **USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 93 (3):p1130-1134 1996
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Plasmodium falciparum malaria parasites were transformed with
plasmids containing P. falciparum or Toxoplasma gondii dihydrofolate reductase-thymidylate synthase (%%%dhfr%%%-ts) coding sequences that confer resistance to pyrimethamine. Under pyrimethamine pressure, transformed parasites were obtained that maintained the transfected plasmids as unrearranged episomes for several weeks. These parasite populations were replaced after 2 to 3 months by parasites that had incorporated the transfected DNA into nuclear chromosomes. Depending upon
the particular construct used for transformation, %%%homologous%%% integration was detected in the P. falciparum %%%dhfr%%%-ts locus (chromosome 4) or in hrp3 and hrp2 sequences that were used in the plasmid constructs as gene control regions (chromosomes 13 and 8, respectively). Transformation by %%%homologous%%% integration sets the
stage for %%%targeted%%% gene alterations and knock-outs that will advance understanding of P. falciparum.

6/3,AB/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09183199 BIOSIS NO.: 199497191569
%%%Homologous%%% recombination and gene replacement at the dihydrofolate
reductase-thymidylate synthase locus in Toxoplasma gondii.
AUTHOR: Donald Robert G K; Roos David S(a)
AUTHOR ADDRESS: (a)Dep. Biol., Univ. Pennsylvania, Philadelphia, PA 19104-6018**USA
JOURNAL: Molecular and Biochemical Parasitology 63 (2):p243-253 1994
ISSN: 0166-6851
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To investigate the feasibility of genomic transgene expression and gene %%%targeting%%% in Toxoplasma gondii, parasites have been transfected with constructs differing in the length of contiguous genomic sequence spanning the dihydrofolate reductase-thymidylate synthase (%%%DHFR%%%-TS) gene. We have previously reported that vectors derived
from a %%%DHFR%%%-TS cDNA 'minigene' containing mutations in the %%%DHFR%%% coding sequence confer pyrimethamine resistance to transfected
parasites (Donald and Roos, 1993). Stably resistant parasite clones arise at high frequency, generally by virtue of transgene integration into parasite chromosomes at locations scattered throughout the genome. In contrast, using a vector which contains 8 kb of contiguous genomic sequence (vs. lt 2 kb for the cDNA-derived vectors), approximately half of the integration events occur by %%%homologous%%% recombination. %%%Homologous%%% recombination appears to occur at even higher frequency
when a 16 kb genomic clone is used. Circular plasmids were more efficient than linearized molecules at producing %%%homologous%%% recombination in
this system, %%%integrating%%% by reciprocal crossing-over to produce a duplication of the %%%DHFR%%%-TS locus. Double crossing-over (or gene
conversion) was also observed at low frequency, resulting in complete allelic replacement in this haploid stage of the parasite. The ability to produce either %%%homologous%%% or non-%%%homologous%%% recombinants, by
the selection of appropriate transformation contructs, has considerable genetic potential.

6/3,AB/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08830170 BIOSIS NO.: 199395119521
Plasticity in chromosome number and testing of essential genes in Leishmania by %%%targeting%%%.
AUTHOR: Cruz Angela K; Titus Richard; Beverley Stephen M(a)
AUTHOR ADDRESS: (a)250 Longwood Ave., Boston, MA 02115**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 90 (4):p1599-1603 1993
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We attempted to generate homozygous %%%dhfr%%%-ts (dihydrofolate
reductase-thymidylate synthase) knockouts in virulent Leishmania major, an asexual diploid protozoan parasite. Transfection of a neo (neomycin phosphotransferase) %%%targeting%%% fragment yielded heterozygous replacement lines with high efficiency. However, second transfections with a hyg (hygromycin B phosphotransferase) %%%targeting%%% fragment in
the presence of metabolites shown to rescue homozygous knockouts in attenuated Leishmania did not yield the expected %%%dhfr%%%-ts-thymidine
auxotrophs obtained previously with attenuated lines. Molecular karyotype, Southern blot, and flow cytometric DNA content analysis of clonal transfectants revealed three classes: (i) genomic tetraploids, containing two wild-type %%%dhfr%%%-ts chromosomes and one neo and one
hyg replacement chromosome; (ii) aneuploid trisomic lines with one wild-type %%%dhfr%%%-ts and one neo and one hyg replacement chromosome;
(iii) diploids bearing %%%homologous%%% integration of the %%%targeting%%% fragment without replacement. Aneuploid and tetraploid
lines predominated. This confirms the common impression that natural populations of Leishmania are often aneuploid. The remarkable ability of these parasites to undergo and tolerate changes in chromosome number suggests a general method for testing whether genes are essential for growth in vitro, as the ability of Leishmania to simultaneously undergo %%%homologous%%% gene replacement while retaining wild-type genes by
increasing chromosome number provides a diagnostic and positive experimental result. Our results show that virulent Leishmania require at least one copy of %%%dhfr%%%-ts and argue that %%%DHFR%%%-TS plays an
unanticipated role in addition to its role in the de novo synthesis of thymidine. These results also have implications for genetic tests of the organization of Leishmania populations.

6/3,AB/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08793987 BIOSIS NO.: 199395083338
Coupling of cytosolic protein synthesis and mitochondrial protein import in yeast: Evidence for cotranslational import in vivo.
AUTHOR: Fujiki Masaaki; Verner Keith
AUTHOR ADDRESS: Dep. Cellular Molecular Physiol., Pennsylvania State University College Med., Hershey, PA 17033**
JOURNAL: Journal of Biological Chemistry 268 (3):p1914-1920 1993
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have utilized a %%%homologous%%% cell-free mitochondrial
protein import system derived from the yeast Saccharomyces cerevisiae, in addition to performing a series of in vivo experiments in yeast, to investigate the coupling between cytosolic protein synthesis and protein transport into mitochondria. We found that the import of bulk mitochondrial proteins was inhibited in both the %%%homologous%%% in vitro reaction and in vivo upon arrest of cytosolic protein synthesis with the addition of cycloheximide. Tight coupling of synthesis and import was also demonstrated in vivo for the beta subunit of the mitochondrial F-1-ATPase. We also investigated the effect of the antifolate methotrexate on the import of a fusion protein consisting of the mitochondrial %%%targeting%%% signal of yeast cytochrome oxidase subunit IV fused to mouse dihydrofolate reductase (the COXIV-%%%DHFR%%%
fusion protein). Methotrexate has previously been shown to inhibit

posttranslational import of COXIV-%%%DHFR%%% by preventing the %%%DHFR%%%
moiety from unfolding. However, we found that antifoliate addition had no inhibitory effect on the import of COXIV-%%%DHFR%%% in vivo, suggesting
that its import into mitochondria in yeast cells occurs cotranslationally. Further, when we treated yeast with the protein ionophore carbonyl cyanide m-chlorophenylhydrazone to collapse the mitochondrial membrane potential and induce the accumulation of extramitochondrial precursor pools, we found that the ability to be imported by a strictly posttranslational mechanism upon reestablishing the membrane potential varied from one precursor to another, suggesting that cotranslational import may be mandatory for the import of some proteins in vivo. In summary, our findings are entirely consistent with the notion that import of proteins into yeast mitochondria occurs cotranslationally under normal conditions in vivo.

6/3,AB/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07772478 BIOSIS NO.: 000092075849
HUMAN NUCLEOPORIN P62 AND THE ESSENTIAL YEAST NUCLEAR PORE PROTEIN NSP1
SHOW SEQUENCE HOMOLOGY AND A SIMILAR DOMAIN ORGANIZATION
AUTHOR: CARMO-FONSECA M; KERN H; HURT E C
AUTHOR ADDRESS: EUROPEAN MOL. BIOL. LAB., P.O. BOX 102209, D-6900
HEIDELBERG, FRG.
JOURNAL: EUR J CELL BIOL 55 (1). 1991. 17-30.
FULL JOURNAL NAME: European Journal of Cell Biology
CODEN: EJCBD
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: NSP1 is an essential nuclear protein in yeast. We observed that anti-NSP1 antibodies label mammalian nuclear pore complexes and recognize
nucleoporin p62. Also peptide antibodies raised against the NSP1 carboxy-terminal end cross-react with p62, a conserved component of the nuclear pore complex in higher eukarytoes. To further analyze the structural and functional similarity between NSP1 and mammalian nucleoporins, we cloned and sequenced nucleoporin p62 from a HeLa cDNA
library. Human p62 consists of a carboxy-terminal domain %%%homologous%%%
to the essential yeast NSP1 carboxy-terminal domain and an amino-terminal half resembling the repetitive middle domain of NSP1. The full-length p62 and a fusion protein consisting of cytosolic mouse dihydrofolate reductase (%%%DHFR%%%) and the p62 carboxy-terminal domain were expressed
in transfected HeLa cells. Only overexpressed full-length p62, but not the %%%DHFR%%%-C-p62 fusion protein, binds wheat germ agglutinin (WGA).
This suggests that modification by N-acetylglucosamine is mainly restricted to the repetitive amino-terminal half of p62 and implies a role of this type of repetitive sequences in nuclear transport. In the transfected HeLa cells, the %%%DHFR%%%-C-p62 fusion protein forms patchy
aggregates that accumulate at the nuclear periphery but are also scattered through the cytoplasm. It is suggested that nucleoporin p62 may be %%%targeted%%% and anchored to the pore complex via its carboxy-terminal domain which reveals a hydrophobic heptad repeat organization similar to that found in lamins and other intermediate filament proteins.

6/3,AB/8 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06235165 BIOSIS NO.: 000086069347
AMPLIFIED EXPRESSION CONSTRUCTS FOR HUMAN TISSUE-TYPE PLASMINOGEN ACTIVATOR
IN CHINESE HAMSTER OVARY CELLS INSTABILITY IN THE ABSENCE OF SELECTIVE PRESSURE
AUTHOR: WEIDLE U H; BUCKEL P; WIENBERG J
AUTHOR ADDRESS: BOEHRINGER MANNHEIM GMBH, DEP. GENET., NONNENWALD 2,
POSTFACH 1152, D-8122 PENZBERG.
JOURNAL: GENE (AMST) 66 (2). 1988. 193-204.
FULL JOURNAL NAME: GENE (Amsterdam)
CODEN: GENED
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: By linking an expression cassette for human tissue-type plasminogen activator (t-PA) to an amplifiable marker gene, its introduction into Chinese hamster ovary %%%dhfr%%%- cells and subsequent
amplication with methotrexate, we have generated cells lines that overproduce the heterologous protein and contain 300-1100 copies of the expression constructs %%%integrated%%% into the hamster genome. We present a detailed investigation of the fate of amplified sequences in the presence and absence of selective pressure by parallel examination of three producer cell lines with respect to relevant parameters. These include the determination of t-PA production upon continuous propagation in culture, the genomic organization of the %%%integrated%%% expression constructs by Southern blotting, and the localization of homogeneously staining regions by in-situ hybridization with biotinylated probes and visualization by interference reflection microscopy. We conclude that in the the three cell lines examined, the decrease in production of t-PA in the absence of methothrexate selection is accompanied by decreases in the number of %%%integrated%%% expression constructs and the size of the amplified regions, whereas all these parameters are stable when selective pressure is maintained. The instability is probably due to the head-to-tail mode of integration of the expression constructs in the hamster genome, which increases the frequency of %%%homologous%%% recombination between the %%%integrated%%% plasmids in recombination-proficient cells in the absence of selective pressure.

6/3,AB/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

05114026 BIOSIS NO.: 000081072150
LOCATION OF AMPLIFICATION DIHYDROFOLATE REDUCTASE GENE ON L-615 CELL CHROMOSOMES
AUTHOR: LIU L; WANG H; FENG S; YANG T
AUTHOR ADDRESS: INST. GENETICS, ACAD. SINICA, BEIJING.
JOURNAL: ACTA GENET SIN 12 (4). 1985 (RECD. 1986). 243-248.
FULL JOURNAL NAME: Acta Genetica Sinica
CODEN: ICHPC
RECORD TYPE: Abstract
LANGUAGE: CHINESE

ABSTRACT: Our experiments demonstrated that there were DMs in part of L615
cells, in other part of L615 cells there were homogeneously staining region (HSR) on chromosomes. The relationship between DM and HSR was little known. But the two forms of abnormal chromosomes are amplified dihydrofolate reductase (%%%DHFR%%%) gene. The L615 cell's resistance to
methotrexate (MTX) resulted from %%%DHFR%%% gene amplification. We
suggest that the cell mitosis in some cases might become DMs. Sometimes the DM is %%%integrated%%% into chromosome during cell mitosis, which
formed a homogeneously staining region. Recently, extracts of cells at various stages of mitosis has been examined with nonionic detergents by steroscopic whole mount electron microscopy. It is revealed that an extensive skeletal framework which is continuous from the plasma lamina to the chromosomes. The granular network occasionally observed in preparations may represent a remanant of the skeletal framework. DM chromosomes enmeshed in this type of highly structured element in conjunction with their close association with the centric chromosomes may present a mechanism for the segregation of these elements during mitosis, assuring their continued presences within the cell population. The HSR chromosome in L615 cells is the eighteenth chromosome. The HSR is located
on the middle size chromosome. The homogeneously staining region showed

strong fluoresence when the cells were stained with Hoechst33258. The hybridization results also show that the HSR is located in a middle size chromosome. Meanwhile the hybridization results demonstrated the nucleotide sequence of HSR was %%%homologous%%% with DM. The dihydrofolate reductase analysis shows that the enzyme quantity is at a high level.

6/3,AB/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

03894262 BIOSIS NO.: 000075072335
INTRONLESS HUMAN DI HYDRO FOLATE REDUCTASE EC-1.5.1.3 GENES ARE DERIVED
FROM PROCESSED RNA MOLECULES
AUTHOR: CHEN M-J; SHIMADA T; MOULTON A D; HARRISON M; NIENHUIS A W
AUTHOR ADDRESS: CLIN. HEMATOL. BRANCH, NATIONAL HEART, LUNG BLOOD INST.,
BETHESDA, MD. 20205.
JOURNAL: PROC NATL ACAD SCI U S A 79 (23). 1982. 7435-7439.
FULL JOURNAL NAME: Proceedings of the National Academy of Sciences of the
United States of America
CODEN: PNASA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Three groups of recombinant bacteriophage containing coding sequences for dihydrofolate reductase (%%%DHFR%%%; tetrahydrofolate dehydrogenase; 5,6,7,8-tetrahydrofolate:NADP+ oxidoreductase, EC 1.5.1.3)
were isolated from 2 human DNA clone libraries. One recombinant (.lambda.hDHFR-1) contains 3 exons that encode the COOH-terminal portion
of human %%%DHFR%%%. The other 2 human %%%DHFR%%% genes (hDHFR-.psi.1 and
hDHFR-.psi.2) lack introns. hDHFR-.psi.2 contains several in-phase termination codons and is only 93% %%%homologous%%% to the normal human
%%%DHFR%%% coding sequences, whereas hDHFR-.psi.1 has an open reading
frame and is virtually identical to the coding sequence of the normal %%%DHFR%%% gene. The region of DNA sequence homology between each
intronless gene and the normal %%%DHFR%%% gene extends 2.9 kilobases
beyond the end of the coding sequences. At the 3' end of this %%%homologous%%% sequence, each intronless gene has an A-rich tract. The
lack of introns and the presence of the 3' A-rich tract suggest that hDHFR-.psi.1 and hDHFR-.psi.2 were derived from processed RNA molecules.
A short DNA sequence, 60 nucleotides 5' to the ATG start codon in .lambda.hDHFR-.psi.2, is directly repeated immediately after the 3' A-rich tract; such terminal direct repeats also flank %%%integrated%%% proretroviruses and transposable DNA elements and are thought to be the hallmark of inserted DNA sequences.

6/3,AB/11 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

04486721 EMBASE No: 1990374830
Gene replacement in parasitic protozoa
Cruz A.; Beverley S.M.
Dept. Biological Chemistry, Harvard Medical School,Boston, MA 02115 United States
Nature (NATURE) (United Kingdom) 1990, 348/6297 (171-173)
CODEN: NATUA ISSN: 0028-0836
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Trypanosomatid protozoa frequently cause severe diseases in humans. Many molecules likely to have a role during the infectious cycle have been identified; yet proof of their function is often lacking. We describe studies in Leishmania major of %%%homologous%%% gene %%%targeting%%%, a
powerful method for testing gene function in other organisms. Following introductionof a construct containing dihydrofolate reductase-thymidylate synthase (%%%dhfr%%%-ts) flanking sequences fused to neomycin phosphotransferase, 45% of the colonies contained the planned %%%homologous%%% replacement; this frequency rose to nearly 100% in transfections using low amounts of DNA. Integrative transfection in Leishmania thus resembles that of Saccharomyces cerevisae in giving predominantly %%%homologous%%% events. To facilitate studies of folate metabolism and chemotherapy the sole %%%dhfr%%%-ts copy in a heterozygous
deletion line was replaced, yielding lines that were functionally %%%DHFR%%%-TSsup -. Although most genes are diploid intrypanosomatids,
methods exploiting the high frequency of %%%homologous%%% recombination
should permit complete replacement of any parasite gene.

6/3,AB/12 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

04211939 EMBASE No: 1990094481
Gene %%%targeting%%% in normal and amplified cell lines
Zheng H.; Wilson J.H.
Department of Biochemistry, Baylor College of Medicine,Houston, TX 77030 United States
Nature (NATURE) (United Kingdom) 1990, 344/6262 (170-173)
CODEN: NATUA ISSN: 0028-0836
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

%%%Targeted%%% recombination in mammalian cells is rare compared with
non-%%%homologous%%% integration. In Saccharomyces cerevisiae the reverse
is true. Differences in %%%targeting%%% efficiency could arise because a %%%target%%% of unique DNA is 200 times more dilute in mammalian genomes
than it is in yeast. We tested this possibility by measuring gene %%%targeting%%% in normal CHO cells with two copies of the dihydrofolate
reductase (%%%DHFR%%%) gene and in amplified CHOC 400 cells, which carry
800 copies. If the concentration of the %%%target%%% gene is critical, amplified cells should show an enhanced frequency of %%%targeted%%% recombination relative to non-%%%homologous%%% integration. Using a positive/negative selection protocol, we demonstrated that the efficiency of %%%targeting%%% into %%%DHFR%%% genes is indistinguishable in normal and
amplified CHO cells. As %%%targeting%%% does not depend on the number of
%%%targets%%%, the search for homology is not a rate-limiting step in the mammalian pathway of gene %%%targeting%%%. Thus, the difference in genome
size is not the basis for the different outcomes of %%%targeting%%% experiments in S. cerevisiae and mammals.

6/3,AB/13 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

03716877 83117693
Intronless human dihydrofolate reductase genes are derived from processed RNA molecules.
Chen MJ; Shimada T; Moulton AD; Harrison M; Nienhuis AW
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec %%%1982%%%, 79 (23) p7435-9, ISSN 0027-8424
Journal Code: PV3
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Three groups of recombinant bacteriophage containing coding sequences for dihydrofolate reductase (%%%DHFR%%%; tetrahydrofolate

dehydrogenase;
5,6,7,8-tetrahydrofolate:NADP+ oxidoreductase, EC 1.5.1.3) were isolated from two human DNA clone libraries. One recombinant (lambda hDHFR-1)
contains three exons that encode the COOH-terminal portion of human %%%DHFR%%%. The other two human %%%DHFR%%% genes (hDHFR-psi 1 and hDHRF-psi
2) lack introns. hDHFR-psi 2 contains several in-phase termination codons and is only 93% %%%homologous%%% to the normal human %%%DHFR%%% coding
sequences, whereas hDHFR-psi 1 has an open reading frame and is virtually identical to the coding sequence of the normal %%%DHFR%%% gene. The region
of DNA sequence homology between each intronless gene and the normal
%%%DHFR%%% gene extends 2.9 kilobases beyond the end of the coding
sequences. At the 3' end of this %%%homologous%%% sequence, each intronless
gene has an A-rich tract. The lack of introns and the presence of the 3' A-rich tract suggest that hDHFR-psi 1 and hDHFR-psi 2 were derived from
processed RNA molecules. A short DNA sequence, 60 nucleotides 5' to the ATG
start codon in lambda hDHFR-psi 2, is directly repeated immediately after the 3' A-rich tract; such terminal direct repeats also flank %%%integrated%%% proretroviruses and transposable DNA elements and are
thought to be the hallmark of inserted DNA sequences.

6/3,AB/14 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0205663 DBA Accession No.: 97-00784
Genetic transformation of the asexual blood stages of the rodent malaria parasite Plasmodium berghei - using a dihydrofolate-reductase and thymidylate-synthase selectable marker, for e.g. recombinant vaccine development (conference abstract)
AUTHOR: van Dijk M R; Janse C J; Waters A P
CORPORATE AFFILIATE: Univ.Leiden
CORPORATE SOURCE: Laboratory of Parasitology, University of Leiden, Leiden,
The Netherlands.
JOURNAL: Am.J.Trop.Med.Hyg. (55, 2, Suppl., 218-19) %%%1996%%%
ISSN: 0002-9637 CODEN: AJTHAB
CONFERENCE PROCEEDINGS: American Society of Tropical Medicine and Hygiene,
45th Annual Meeting, Baltimore, MD, 1-5 December, 1996.
LANGUAGE: English
ABSTRACT: 2 Stable drug selectable transfection systems were developed for
asexual blood stages of Plasmodium berghei, for use e.g. in vaccine development. Both systems were based on use a gene encoding drug resistant %%%homologous%%% dihydrofolate-reductase (EC-1.5.1.3)-thymidy
late-synthase (EC-2.1.1.45) (%%%DHFR%%%/TS) as a selectable marker. A
stable transfection system was developed, where vectors were episomally maintained to a maximum copy number of 15/nucleus during drug pressure. Expression of the plasmid copy of the %%%DHFR%%%/TS gene was
proportional to the observed copy number. A stable transfection system was developed, where the vector, with a %%%target%%% nontranscribed
subtelomeric repeat sequence specific for P. berghei, %%%integrated%%%
in the genome in a site-directed manner, in subtelomeric regions of different chromosomes by %%%homologous%%% recombination via a single
reciprocal crossover event. %%%Integrated%%% DNA was stable after 70
generations without drug pressure. Reverse transcription-polymerase chain reaction and hybridization showed that the %%%DHFR%%%/TS gene was
expressed, indicating that malaria chromosome subtelomeric can support transcription. (0 ref)

6/3,AB/15 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0121458 DBA Accession No.: 91-09100 PATENT
Protein production by culturing recombinant mammalian cells - gene amplification in human skin fibroblast cell and dihydrofolate-reductase-deficient CHO cell culture
PATENT ASSIGNEE: Cell-GeneSys %%%1991%%%
PATENT NUMBER: WO 9106667 PATENT DATE: 910516 WPI
ACCESSION NO.:
91-164214 (9122)
PRIORITY APPLIC. NO.: US 432069 APPLIC. DATE: 891106
NATIONAL APPLIC. NO.: WO 90US6436 APPLIC. DATE: 901106
LANGUAGE: English
ABSTRACT: A method for production of a mammalian recombinant protein (I) is
claimed. An amplifiable gene (AG) is introduced by %%%homologous%%%
recombination in juxtaposition to a %%%target%%% gene (TG), the resulting combination transferred to a host and the TG amplified by means of the AG. The resulting host may then be grown in culture with enhanced expression of the TG. Also claimed are human cells containing an AG which is at other than its wild-type site in the human genome and is within the locus of a protein-encoding TG, to provide amplification of the TG; and ii. non-human mammalian cells for the production of mammalian proteins in culture, containing an amplifiable region comprising an AG within 10 kb of a protein-encoding human wild-type gene, where the 2 genes are separated by a human wild-type sequence associated with the TG and a flanking sequence associated with the AG. More specifically, the primary cells are human diploid skin fibroblasts. The secondary cells are dihydrofolate-reductase (%%%DHFR%%%, EC-1.5.1.3)-deficient CHO cells. The AG is a %%%DHFR%%%
gene. The DNA fragments are metaphase chromosomes or restriction fragments. (34pp)

6/3,AB/16 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0121457 DBA Accession No.: 91-09099 PATENT
Methods for expression of mammal genes - large-scale recombinant protein production, gene DNA amplification after %%%homologous%%% recombination; mutant dihydrofolate-reductase DNA cassette expression in human diploid fibroblast
PATENT ASSIGNEE: Cell-GeneSys %%%1991%%%
PATENT NUMBER: WO 9106666 PATENT DATE: 910516 WPI
ACCESSION NO.:
91-164213 (9122)
PRIORITY APPLIC. NO.: US 432069 APPLIC. DATE: 891106
NATIONAL APPLIC. NO.: WO 90US6425 APPLIC. DATE: 901106
LANGUAGE: English
ABSTRACT: High yields of mammal proteins are prepared by culturing normal
or tumorous mammal secondary host cells containing multiple copies of an amplifiable region encoding a selectable marker (pesticide resistance gene) and containing a %%%target%%% gene heterologous to the
secondary expression host and encoding a desired protein. The secondary host cells are prepared by transformation of primary mammal, preferably human fibroblast, cells containing a %%%target%%% gene with a construct
comprising an amplifiable gene (preferably mutant dihydrofolate-reductase (%%%dhfr%%%, EC-1.5.1.3) with increased Km) and
at least 1 flanking region of total length 150 bp %%%homologous%%% with, and preferably within 50 kb of, DNA at the locus of the %%%target%%% gene. The amplifiable gene is inserted at a site where it does not affect expression of the %%%target%%% gene. DNA fragments
containing the amplifiable region are isolated from selected primary cells and used to transform secondary hosts, which are %%%dhfr%%%
-deficient. Transformed human cells, and a method for transforming the cells are claimed. The method is useful for large gene cloning without

cloning mRNA. (31pp)

6/3,AB/17 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0102145 DBA Accession No.: 90-04836
Tapping the cellular telephone - comparison of gene %%%targeting%%% frequency and function in mammal and yeast cell; potential transgenic mouse construction
AUTHOR: Capecchi M
CORPORATE SOURCE: Howard Hughes Medical Institute, University of Utah, 337
South Biology, Salt Lake City, Utah 84112, USA.
JOURNAL: Nature (344, 6262, 105) %%%1990%%%
CODEN: NATUAS
LANGUAGE: English
ABSTRACT: Gene %%%targeting%%% facilitates the construction of transgenic
mice of virtually any desired genotype. Cloned DNA is mutated, introduced into a pluripotent stem cell derived from a mouse embryo, and the transformed cells are introduced into mouse blastocytes by microinjection. Interbreeding yields animals homozygous for the desired mutation. The efficiency of %%%targeting%%% into the dihydrofolate-reductase (%%%DHFR%%%, EC-1.5.1.3) gene was identical in
a normal and in an amplified Chinese hamster ovary cell line containing 400 copies of the %%%DHFR%%% gene. In yeast, gene %%%targeting%%%
frequency is proportional to the number of %%%target%%% sequences in
the genome. The gene %%%targeting%%% frequency depends more on the
extent of homology between the exogenous and chromosomal sequences in
mammal cells than it does in yeast. In yeast this dependence is linear, while it is exponential in mammal cells. %%%Homologous%%% ends in
%%%targeting%%% vectors are more critical for gene %%%targeting%%% in
yeast than in mammal cells. Yeast mediates almost exclusively %%%homologous%%% recombination events, unlike mammal cells. The gene
%%%targeting%%% machinery is designed for DNA communication within a
cell and for DNA repair. (10 ref)

6/3,AB/18 (Item 5 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0082289 DBA Accession No.: 89-00280
Amplified expression constructs for human tissue-type plasminogen activator in Chinese hamster ovary cells: instabiltiy in the absence of selective pressure - CHO cell culture, transfection, methotrexate selection
AUTHOR: Weidle U H; Buckel P; Wienberg J
CORPORATE AFFILIATE: Boehr.Mannheim
CORPORATE SOURCE: Boehringer Mannheim GmbH, Department of Genetics,
Nonnenwald 2, Postfach 1152, D-8122 Penzberg, Germany.
JOURNAL: Gene (66, 2, 193-203) %%%1988%%%
CODEN: GENED6
LANGUAGE: English
ABSTRACT: By linking an expression cassette for human tissue plasminogen
activator (t-PA) to an amplifiable marker gene (in plasmid pSVtpA-%%%dhfr%%%), its introduction into CHO %%%dhfr%%%- cells and subsequent
amplification with methotrexate, cell lines were obtained that overproduced the heterologous protein and contained 300-1100 copies of the expression constructs %%%integrated%%% into the hamster genome. CHO
%%%dhfr%%%- cells were transfected with plasmid pSVtpA-%%%dhfr%%%
precipitates. 3 Stable transformants were scored and detailed investigations are presented of the fate of amplified sequences in the presence and absence of selective pressure. In the 3 cell lines, the decrease in t-PA production in the absence of methotrexate selection was accompanied by decreases in the number of %%%integrated%%%
expression constructs and the size of the amplified regions; these were stable in the presence of selective pressure. Instability was probably due to head-to-tail mode of integration of the expression constructs in the hamster genome, which increases the frequency of %%%homologous%%%
recombination between %%%integrated%%% plasmids in recombination-proficient cells in the absence of selective pressure. (36 ref)

6/3,AB/19 (Item 6 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0081856 DBA Accession No.: 88-12705
New genetic methods for mammalian cells - including nonsense suppression, controlled amplification and gene %%%targeting%%% by %%%homologous%%%
recombination
AUTHOR: Sedivy J M
CORPORATE SOURCE: Department of Molecular Biophysics and Biochemistry, Yale
University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, USA.
JOURNAL: Bio/Technology (6, 10, 1192-96) %%%1988%%%
CODEN: BTCHDA
LANGUAGE: English
ABSTRACT: Several recent developments may facilitate the manipulation of
genes in mammalian cells. Mutational analysis using nonsense suppression has the advantages of generating tight, null phenotypes due to premature termination of encoded polypeptides, and low reversion frequencies due to the low probability of intragenic suppression. The procedure for the isolation of amber-suppressing cell lines is outlined. The amplification resulted in suppression levels of 50% readthrough of amber codons. The behavior and morphology of the resulting cells is discussed. Gene regulation using rapid amplification is a method for boosting protein production. One technique is based on in situ chromosomal gene amplification, using the dihydrofolate-reductase (%%%dhfr%%%, EC-1.5.1.3) gene. The method can
be adapted for the overproduction of a variety of protein products and has several advantages over the commonly used %%%dhfr%%% amplification
method. %%%Targeted%%% %%%homologous%%% recombination of transfected
DNA into the mammalian genome would allow any cloned open reading frame
to be taken up and directly disrupt the corresponding locus in the chromosome. (43 ref)
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